THE UNIVERSITY OF ALBERTA

EVALUATION OF pH, TOTAL SUGARS AND RELATIVE AMOUNTS OF MALATE AND CITRATE AS CRITERIA FOR EARLINESS IN TOMATOES

by

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ABSTRACT

This thesis reports on the correlation between the acid content of Lycopersicon esculentum L. and the time of ripening.

There were three major objectives to the study:

(1) To determine which stage of fruit development gives the most significant correlation between earliness of the tomato and the changes in malic and citric acid.

(2) To determine the pH, the total sugar percentage, the malic and citric acid in densicord units and the malic acid, citric acid ratio of tomato stocks classified as early, mid-season and late maturing under Alberta climatic conditions.

(3) To ascertain within a population of a promising seedling, how suitable these determinations might be in selecting for earliness.

Red Bobs, Earlicrop and Starfire were grown in the first phase of the investigation and the fruits were harvested at seven stages of fruit development. Detailed analyses of the three varieties suggested that changes in pH, total sugar, malic and citric acids were more useful indicators of earliness in: the final fruit development stage (C), the green ripening stage (D), the early colored ripening stage (E) and the late colored ripening stage (F) than in the other stages of fruit development studied.

Twenty varieties were grown in the second phase of the investigation. They were harvested at four stages of fruit development. The pH, total sugar percentage and the relationship of malic and citric acid was useful in distinguishing between early and late varieties. Koch stated in his work that a shorter growing period is dependent on the

presence and quantity of malic acid. The importance of malic acid to the earliness of the tomato is recognized by the author. The study strongly indicates however that the relationship between earliness of tomato varieties and the biochemical changes in their fruit is a more complicated matter than suggested by Koch. On the basis of results obtained it is suggested that the following factors are important in selection for earliness in the tomato:

An early peak in malic acid content.

A high densicord reading for malic acid in comparison with citric acid.

The disappearance of malic acid in the late colored ripening stage of fruit development.

An early peak in the total of the two acids. An early peak in citric acid.

Continuous decrease in the citric acid during ripening.

Thirteen selections of a promising seedling (B.V. 132-2116-3) were grown in the third test. They were harvested in four stages of fruit development. The progeny of selection 2116-3 chosen on the basis of visual earliness in 1965 showed a large variation in earliness in the 1966 season. Visual earliness (e.g. fruit color) was not a reliable method in selecting for earliness because of the human factors involved in the selection.

Although the detailed analyses of malic and citric acids were a lengthy procedure, the pattern of the acid relationship in most cases showed a reliable correlation with the actual earliness of the selections.

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I am indebted to my wife, Magdi, for her encouragement throughout the course of this study and dedicate this thesis to her and our sons, Steve and George, whose patience and understanding made this study possible.

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I. INTRODUCTION

The tomato, Lycopersicon esculentum L. has as a food shown spectacular development during the past 100 years. It is one of the most important 'protective foods', because of its special nutritive values and its widespread production and consumption.

The nutritive value of the tomato is due primarily to its vitamin content. The most abundant vitamin in the tomato is ascorbic acid or vitamin C. The second most important is vitamin A. Vitamin A in turn is followed by lesser amounts of niacin (B_2) and thiamin (B_1). One medium size tomato, will, on the average, supply 45% of the vitamin C recommended as the dietary allowance for an average physically active man for one day. It would also supply 33% of the vitamin A, 6% of the niacin, 6% of the thiamin and 7.5% of the iron allowance.

The increase in the per capita consumption of tomatoes and tomato products during the last 30 years represents a marked improvement in the average Canadian diet. A further increase in the consumption of tomatoes would further improve the nutrition of our population

Production of the tomato in Canada has increased remarkably. The 1966 production was 352,037 tons on 29,770 acres. Of this total 23,230 acres or 303,139 tons were contracted for processing. The processed products were mainly soups, juices and ketchups.

Canadian production supplied only part of our consumption. Approximately 92,742 tons of tomatoes and 42,396 tons of tomato products were imported to Canada during the same year. The total farm value of Canadian production in 1966 was $19,398,000 and the tomato imports represented an expenditure of $30,166,000. The importance of the tomato in the

food industry of Canada justifies the concentrated efforts of our plant breeders on the development of new tomato varieties, adapted to more northern growing conditions.

Due to the relatively short growing season in Alberta, earliness is one of the primary objectives of tomato research programs in this area.

Assessing earliness, thus, becomes an important problem in a research program. Tomato researchers use many methods of determining earliness, singly or in combination. Some examples of such criteria are:

a) number of days to first flower development.

b) number of days to set of the first fruit cluster.

c) number of days to ripening of the first fruit.

d) number of days to ripening of the first three fruits.

e) number of days to the first harvest.

There are certain advantages and disadvantages to each of these methods, but they are all far from adequate due to the subjectivity of human factors involved, or because of the effect of climatic variations between seasons. An objective method, practical enough to handle a great number of samples in a short period of time would be of considerable value in determining hereditary earliness.

Paper chromatographic acid determination might serve as a tool for measuring earliness in tomatoes.

The objectives of this work were to determine the potential of any relationship between friot acod and earliness in a series of varieties and seedlings as a means of selecting for earliness and to determine whether correlations exist between pH and earliness and total

sugar and earliness in these stocks. The interrelations of all of these characteristics had to be given special consideration in the interpretation of observations.

II. REVIEW OF LITERATURE

A. THE ROLE OF PLANT ACIDS IN PLANT METABOLISM

1. Plant acids and photosynthesis

Plant acids act as important intermediates of photosynthesis.

Several organic acids are the early products of photosynthesis, according to Burris (14). Malic acids may not be in the main path of photosynthesis, but the key importance of phosphoglyceric acid is now generally accepted.

One of the most important recent developments in the knowledge of photosynthesis has been the discovery of the coupling of light energy for effecting certain model carboxylation reactions which results in the synthesis of organic acids.

2. Plant acids and respiration

Organic acids accumulate in plants during the night and disappear during the day.

It has been suggested that organic acids break down during the day, yielding CO_2 which is utilized in photosynthesis, while during the night, increased amounts of CO_2 in the cells due to respiration, bring about the incorporation of CO_2 in organic acids. Bonner and Bonner (9), reported experiments in which they fed $C^{14}O_2$ to Bryophyllum leaves and observed its incorporation into organic acids. Low temperatures and darkness promoted synthesis of organic acids, while higher temperatures and light depressed it.

It has been inferred from observations on changes in the respiratory quotient (R.Q.) of experimental material, that organic acids serve as a respiratory substrate. Thus Bennet-Clark and Bexon (7)

observed that beetroot discs, when immersed in solutions of malate, citrate and succinate, not only showed an increase in their respiration, but their R.Q. was between 1.5 and 2.3. When supplied with sugars, their R.Q. dropped to 1. In other cases disappearance of organic acids has suggested their oxidation. Thus Vickery (48) observed the disappearance of malate from detached tobacco leaves kept in darkness for 48 hours, and concluded that malate served as a respiratory substrate. Ulrich (47) noticed changes in the organic acid fraction of excised barley roots during salt accumulation, and thought that organic acids may be broken down to supply energy when anions are observed in excess of cations.

In the formulation of a picture of the role of organic acids in respiration, the paper by Krebs (28), ushered in a new concept. He suggested that the hub in respiratory metabolism is represented by the cyclic oxidation of a number of organic acids, with subsequent passage of the removed hydrogen to oxygen. Consequently, there is a disappearance of organic acids, production of CO_2 and consumption of oxygen. Since citric acid and other tricarboxylic acids participate in such a cycle, it has been called by various workers the Krebs, citric acid or tricarboxylic acid cycle.

As the Krebs cycle does operate in plants, one would expect that an addition of various Krebs cycle intermediates should increase the rate of respiration. In potato slices respiration was increased upon the addition of citrate, α-ketoglutarate, succinate and acetate as reported by Barron, et al. (4); in spinach leaves upon the addition of succinate, malate, fumarate, isocitrate, and citrate according to Bonner and Wildman (11); in tobacco leaves upon the addition of acetate, citrate, cis-aconitate,

succinate, fumarate, malate and oxalosuccinate as shown by Burris (14); and in tomato root tips upon addition of fumarate, succinate, citrate and oxalacetate, as reported by Henderson and Stauffer (20). Similar increases in respiration upon the addition of various Krebs cycle intermediate have been observed in bean leaves by Johnson and Hoskin (23), rhubarb leaves by Morrison (35) and carrot slices by Turner and Hanley (46).

Rationing of $CH_3C^{14}O\ OH$ to tobacco, according to Krotkov, et al. (29), and to tomato, corn, flax and castor bean leaves, as reported by Hoskin, et al. (21), resulted in the emission of $C^{14}O_2$ indicating that acetate served as a respiratory substrate.

Other reactions reported for tomato stem slices indicated the splitting of malic acid to glycolic acid, the oxidation of the glycolic, glyoxylic and formic acids and the oxidation of amino acids. The rate of respiration of tomato stem slices varied considerably as resported by Link, et al. (31). The highest values were obtained from plants in good nutritional state, and the lowest in a starved plant. The R.Q. of 1.0 remained constant.

Glucose fermentation was found to follow both glycolytic and alcohol fermentation pathways, the ratio of ethyl : alcohol : lactic acid being 6:6:1. Fermentation seems to take place according to the Embden-Mayerhof scheme. In the presence of oxygen there was no formation of alcohol or lactic acid.

Pyruvate added to tomato stem slices was metabolized by direct oxidation of acetic acid and by dismutation of lactic and acetic acids and CO_2. The metabolism of acetic acid was demonstrated by its condensation with oxaloacetic acid to form citrate.

The presence of aconitase, of isocitric dehydrogenase, of succinic dehydrogenase, and malic dehydrogenase, as well as the inhibition of respiration by malonic acid, favor the hypothesis that oxidation of carbohydrate in tomato stem slices proceeds via the citric acid cycle.

The organic acids are readily interconverted in many plant tissues. The production of citrate from malate in tobacco leaves kept in darkness is a classical example of such a change. The citrate stimulated respiration of the leaves and an excess of the citrate taken up was respired or converted to other compounds. Whereas, in the light, malic acid derived twice as high a specific activity as citric acid from labelled glycolate, and in the dark the specific activity of citric acid was four times that of malate. This result is compatable with the suggestion that a tricarboxylic acid cycle active in the dark, may be suppressed in light.

Braunstein (12) reports on the central position of organic acids in respiratory hydrogen transport.

Studies on malonate inhibition of respiration have contributed much to the understanding of the plant's respiratory mechanism. It should be noted that inhibition of respiration by malonate is observed only at pH 5.0 or below according to Bonner and Varner (10), as malic acid appears to enter the cell membrane at an appreciable rate only as the undissociated acid or as the monovalent ion.

3. <u>Plant acids and the formation of carbohydrates, proteins and fats</u>

The tricarboxylic acid cycle releases energy and interrelates fat, carbohydrate and protein metabolism according to Burris (14).

Many workers have stressed the importance of organic acids in nitrogen metabolism, and there has been an excellent review on the subject.

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Braunstein (12) presents a diagram of various metabolic processes showing the role of organic acids in the formation, interconversion and breakdown of amino acids.

As Thimann and Bonner (43) state, "it is the group of substances we refer to as organic acids that occupy the central and therefore the key position of the carbohydrate, protein and fat metabolism in plant cells".

4. Plant acids and growth

The metabolism of organic acids has a close relationship to growth according to Thimann and Bonner (43). While the exact nature is yet far from clear, such a relationship has been demonstrated at low concentrations of acids and evidently involves their oxidative metabolism.

Cameron's studies (15) on germinating peas have indicated an exceedingly active tricarboxylic acid cycle, which is of primary importance in the metabolic reactions of germination.

Organic acid action is less when the growing parts of the plant become older. The variation of effect with age can be correlated with changes in organic acid content. Sensitivity to iodoacetate increases markedly with the age of the coleoptile. The malate, citrate and total organic acids decrease steadily with age. The organic acids are natural protective agents against iodoacetate. An inhibiting effect at a high concentration may not be in conflict with a stimulating effect at a low concentration.

5. Plant acids and the Krebs cycle

Acids of the Krebs cycle belong to the group of plant acids, and as their name implies, they are of wide distribution in plants. Literature on the occurrence of these acids in the plant kingdom has been carefully tabulated by Thimann and Bonner (43). Citric, isocitric and malic

acid are probably ubiquitous in plant tissues. Succinic and fumaric acids are of frequent occurrence and cis-aconitate, α-ketoglutarate and oxalacetate have been reported in plant tissues.

Some of the Krebs cycle acids in plant tissues may be present in very high concentrations. Most commonly citrate or malate accumulate but isocitrate, cis-aconitate or fumarate may do so in particular instances. These high concentrations are maintained in the vacuole and the acid concentrations of the surrounding cytoplasm are quite low. While it is clear that the principal acids of the cycle are found throughout the plant kingdom, the occurrence of all these together in a single plant is difficult to demonstrate. All enzymes of the cycle have been shown to occur in plants and the reported occurrence of the various enzymes has been summarized by Krebs (28). Until recently not all the enzymes had been detected in one tissue. James (22) has tabulated the tissues in which malic acid and isocitric dehydrogenases have been shown to occur. Malic dehydrogenase and isocitric dehydrogenase were readily detected in many tissues.

There have been numerous demonstrations that plant tissues can metabolize the acids of the Krebs cycle; beetroot, Bennet-Clarke and Bexon (7); spinach leaf, Bonner and Wildman (11); potato tuber, Barron, _et al_. (4); rhubarb leaf, Morrison (35); and tomato stem, Link, _et al_. (31).

The paper of Millerd (34), constitutes an important step in the study of the Krebs cycle in plants because, in a particular enzyme complex from a single tissue, the integrated cycle was demonstrated.

B. THE SUGAR CONTENT OF THE TOMATO

A refractive index may be used as a means of calculating total solids in tomato pulp. Various investigators have found a direct

relationship between the refractive index and the amount of total solids present (5, 8, 33, 38).

Total solids content of tomatoes of a given variety grown in a cool, foggy climate was higher than for the same variety grown in a warmer and drier climate according to Smith (40). The higher the summer temperature, the lower was the content of total solids. A low water supply in the soil increased the dry matter (D.M.) of the fruits. Tomatoes from different locations on a single plant varied markedly in D.M.

The total solids of the tomato consist principally of carbohydrates, particularly reducing sugars. Various reports indicate variations in reducing sugar content from 2.3 to 4.5 percent on fresh weight basis, and an average of about 3.5 percent.

The reducing sugar content increases during ripening. Beadle (5), found that the first fruits to ripen had the highest sugar content and that premature picking resulted in a lowered sugar content, even though the fruit subsequently ripening off the vine. Studies by Smith (40) showed that invert sugar increased or remained high when the temperature was high and decreased slightly following rain. He reported a significant correlation between temperature, expressed in degree days, and invert sugar of Marglobe tomatoes.

The tissues of the core of the fruit have been reported to contain relatively high proportions of reducing sugars, total solids and acid hydrolyzable matter, but the locular material was lower in amounts of these constituents than any other part of the fruit. Most reports agree that the sucrose content of tomatoes is very low, probably in most cases below 0.50 percent on the fresh weight basis, although Smith (40), reports that it may reach as high as 1.5 percent.

C. THE ACIDITY OF THE TOMATO

The tomato is strongly acid. Smith (40) reports a range of pH of from 4.06 to 4.60; Bohart (8) from 4.29 to 4.59; Saywell and Crues (39) from 3.8 to 4.4 and Rosa (38) from 4.1 to 4.5. The usual pH range of tomatoes is between 4.0 and 4.5 according to Thompson (44).

Although the range of pH fround in tomatoes is not great, numerous reports indicate variations in total acidity. Rosa (37) and Thompson (44), report that during the ripening process there is a decrease in total acidity. According to Smith (40) the acidity decreased when the temperature was high and increased immediately following rainfall. Drought conditions resulted in higher acid content of tomato fruits when compared with plants supplied with a normal amount of water. The acid content was high at the beginning of the season, low in the middle and high again at the end. Bohart (8) reports that green tomatoes are somewhat more acid than partially or fully ripe ones, but there were some indications of a slight increase in acidity in the fully matured fruit as compared with the partially colored fruit. Saywell and Coness (38) report a consistant difference in total acid between several varieties and a wide range of variation between the fruits of individual plants of a given variety. Thompson (45) reported that the locular material was higher in total acid than were the cores or walls. The acidity was caused chiefly by citric acid and malic acid.

D. THE RELATION BETWEEN THE GEOGRAPHICAL DISTRIBUTION OF FRUITS AND THE DEVELOPMENT OF SPECIFIC PLANT ACIDS

It has become apparent that there is a correlation between the characteristic and dominant acid of a fruit and the geographic zone in

[illegible]

which it normally grows, or between the appearance of certain carboxylic acids and temperature. The most common acids of fruits are malic acid, tartaric acid and citric acid.

Koch (25) reported that plants which contain malic acid, used the acid for respiration at 12° C. Tartaric acid was used in respiration at 20° C and with citric acid 30° C was needed for the plant to utilize the acid for respiration in the presence of sugar. Therefore, acid content of the ripening fruits decreases significantly only above certain specific temperatures.

The R.Q. of the plant is generally 1. The production and utilization of acids in acid rich fruits is closely correlated with gas exchange according to Koch (25). It was shown in his observations that the intensity of respiration was increased at 33° C as long as there was available a significant amount of acid in the plant. The R.Q. rises above 1 in proportion to the acid content of the fruit. Also the relation between the decomposition of acids and temperature is strongly influenced by the type of acid.

1. Fruits in which malic acid predominates

Those fruits in which malic acid predominates, are grown in the temperate zone, according to Paech and Tracey (36). However, providing that the average daily temperature is above 5° C for 170 days of the year, apples can be grown further north than the temperate zone, as reported by Koch (25).

2. Fruits in which tartaric acid predominates

On the basis of data supplied by Pálinkás (37), the tartaric acid containing plants, for example, the grape, are the habitants of a

zone where the average temperature is 9 - 21° C. Grapes cannot be grown above 52° latitude because the fruit would not ripen. If the plant breeders could produce a grape variety, which would contain malic acid instead of tartaric acid, it would be possible to extend the growing zone of the grape north of the 52° latitude, according to Paech and Tracey (36).

3. Fruits in which citric acid predominates

The growing of those fruits which contain mainly citric acid, such as lemon, orange and grapefruit, etc., is limited to the Mediterranean and subtropical territories where average daily temperature is sufficiently high for the utilization of citric acid.

F. THE PLANT ACIDS OF THE TOMATO FRUIT

Previous observations indicate that there is a correlation between the quality and the quantity of carboxylic acids which are present in plants at the time of ripening. Koch (25), reported such a correlation between citric and malic acid content of the tomato fruit and temperature and maturity. Characteristically the tomato fruit contains both malic and citric as the main acids, and some other acids in smaller proportion. A short review of these acids is presented as follows:

1. Malic Acid

$$\begin{array}{l} COOH \\ | \\ CHOH \\ | \\ CH_2COOH \end{array}$$

Malic acid appears to be ubiquitous in higher plants. It occurs in the fermentation of glycerol and in the aerobic utilization of acetic and pyruvic acids and ethanol (18). Malic acid is utilized in the formation of propionic and acetic acids and carbon dioxide. It may function as a catalyst in the dissimilation of glycerol and glucose, an interaction

involving phosphorylation according to Barker and Lipman (3). Malic acid in many respects behaves like fumaric acid. Oxygen is taken up by propionic acid bacteria in the presence of malic acid as reported by Stone, *et al*. (41), indicating a dehydrogenase. Malic acid is reduced readily to succinic acid according to Krebs (28).

2. Citric Acid

$$\begin{array}{ccccc} & & CH_2COOH & & \\ & & | & & \\ HO & — & C & — & COOH \\ & & | & & \\ & & CH_2COOH & & \end{array}$$

Martin (32) reports that the biochemical role of citric acid was first demonstrated in sucrose culture of *Citromyces pfefferianus* and *C. glaber*. Because of the industrial importance of citric acid, the mechanism of its formation by fungi has received much attention.

3. Minor Acids

There are some other acids also present in the tomato in much smaller quantities according to Koch (25).

Oxalic Acid: the presence of oxalic acid in fruit was also reported by Buch (13).

Tartaric Acid: Swaby (42) also found tartaric acid in tomato fruit. Tartaric acid was first obtained from grape tartar. More recently the acid has been reported by Crombie (18) to be present in a number of fruits.

The metabolism of tartaric acid has been studied particularly in viniculture. Malic and tartaric acids account for most of the total acids of the grape, although small amounts of citric, tannic, and phosphoric acid also occur, as reported by Amerine and Winkler (2). During ripening both malate and tartrate decrease according to Amerine (1) and Vitte and

Guichard (49). This decrease is more marked at high temperature as observed by Flanczy (19).

Chlorogenic Acid and Aconite Acid appear in fruit, leaf, and root, also reported by Buch (13). He also observed the appearance of the following acids:

Isocitric Acid

Succinic Acid in fruit, leaf and stalk

Glycolic Acid in leaf

Lactic Acid in fruit and juice of fruit

Salicylic Acid

A relation between the malic and citric acid content of the tomato fruit and the time needed to ripening was reported by Koch (26).

F. BREEDING FOR EARLINESS

Previously tomato breeders have measured only the total acid content of the tomato fruit, because the selection and analysis of the different acids was a time-consuming and tedious job. The more detailed analysis of individual acids was not practical in breeding work, when thousands of samples have to be analysed in a peak period of the growing season. Recent work by Koch (25) indicates that chromatographic acid separation and determination is suitable for the quick testing of a high number of units in a relatively short period of time.

Koch (27), analyzed a large number of tomato varieties for malic and citric acid content. He was particularly interested in the quantity and ratio of these two main acids in different tomato varieties, and how the time of ripening was influenced by the distribution of the two acids. He also wished to determine whether there were any individual

plants in a population which contained more malic acid than citric acid.

In his investigations the quantity and ratio of malic acid and citric acid were measured by paper chromatography. The analysis and calculation of the correlations were conducted on fruit of 18 tomato varieties, which were in the early colored ripening stage of fruit development. He states that on the basis of his results a shorter ripening period is dependent upon the presence and quantity of malic acid.

This correlation indicates that one method for the breeding of earlier ripening tomato varieties could be the development of new lines with high malic acid content. This breeding problem might be approached in several ways.

1. The most simple method would be to make selections from a population of plants, which have a high malic acid content.
2. Another method could be to cross parent varieties with medium malic acid content, if the genotype of the parental factors is different:

A1 A1 a2 a2 medium malic acid	X	a1 a1 A2 A2 medium malic acid
	A1 a1 A2 a2 medium malic acid	
A1 A1 A2 A2 high malic acid		a1 a1 a2 a2 low malic acid

3. A further method of breeding might be to select tomato varieties in which we can find malic acid in all stages of fruit development.

According to Koch (26), in the case of tomato, the number of days required to ripen fruit can be decreased either by increasing the quantity of malic acid present in the fruit, or by decreasing the quantity of citric acid.

G. FRUIT BREEDING FOR FLAVOR

The quantitative changes in plant acids and the presence, appearance and disappearance of different kinds of acids also gives valuable information in fruit breeding for flavor.

Flavor is a complex characteristic in which sugars, salts, bases, aromatic compounds and organic acids are important components. The analysis of the acids helps in a more accurate determination of flavor characteristics.

Jorden, et al. (24), applied the technique successfully in breeding of berries and currants.

III. GENERAL EXPERIMENTAL PROCEDURE

The procedures outlined in this portion of the thesis apply to all of the experiments.

Specific procedures, related only to one or some of the experiments are outlined under Part One A, Part Two A and Part Three A.

All of the bedding plants were seeded in the greenhouse on the 20th of April and were field set in the first week of June.

A. Stages of Harvesting

The levels of citric and malic acid, pH and total sugar content of the tomato fruits were tested at seven stages of fruit development:

Stage A. Early fruit development - when the fruits were up to 30% of the size of the fully grown fruit.

Stage B. Medium fruit development - when the fruits were 31 - 60% of the size of the fully grown fruit.

Stage C. Late fruit development - when the fruits were 61 - 100% of the size of the fully grown fruit.

Stage D. Green ripening - when the locular cells were completely filled. Jelly had already developed but the color of the jelly was still green. The fruits were solid, and suitable for very distant markets. Careful post-harvest ripening was needed.

Stage E. Early colored ripening - when the greater area of the fruit surface had a pinkish or light red color. The fruits were firm and suitable for not too distant markets.

Stage F. Late colored ripening - when the characteristic color of the variety was fully developed. Fruits were still firm, post harvest ripening was not needed. The fruits were suitable for local market, canning, juicing and pureeing.

Stage G. Dead ripen or over-ripe - when the fruits had become a dark red color and were soft. They were not suitable for the fresh market or canning, but could still be used for juicing.

The samples were always harvested beginning at 8:00 a.m. Fruits uniformly shaded by foliage were harvested and carefully selected for uniformity of color in each different stage of fruit development.

Immediately after harvesting in their respective ripening periods, all samples were washed, air dried and then quick frozen in polyethylene bags at -20° C temperature and stored in the same freezer.

B. Total Sugar Determination

Total sugars were determined by using the method described by Lepper, H.L. et al. (30). The frozen samples were thawed overnight at 20° C. The sample was then blended for one minute in a Waring Blender and strained through double cheesecloth.

A 250 ml beaker was balanced on the scale and 75 g of the cleared tomato juice were weighed into the beaker. Distilled water was added until the beaker was 2/3 full and a thermometer was placed in the sample. Samples were placed in a steam bath and cooked for 1 hour at a temperature of 85° C.

Samples were then washed into a 250 ml volumetric flask, cooled to room temperature and made up to volume.

The contents were shaken for 30 seconds and then filtered through Watman No. 1 filter paper.

Ten ml of the filtrate and 5 ml of HCl were then pipetted into test tubes. The test tubes were plugged by rubber stoppers fitted with capillary tubes.

The samples were then heated in a water bath at 95° C for 15 minutes and then cooled in a water bath to room temperature.

Subsequent to cooling the samples were neutralized to pH 7 with 6 N NaOH (about 5.9 ml), poured into a 100 ml volumetric flask and brought to 100 ml volume. From the 100 ml flasks 15 ml were taken and made to volume in a 50 ml volumetric flask.

Five ml of solution A* and 5 ml properly diluted solution were placed in large test tubes, which in turn were placed in boiling water for 15 minutes. After boiling the test tubes were cooled in a water bath. Then 1 ml of 5 N H_2SO_4 was added and the contents were shaken for half a minute. Samples were allowed to stand for one and a half minutes with stoppers in the tubes. 0.5 ml of starch solution was then added and titration took place immediately using 0.005 N $Na_2S_2O_3$.

The differences between the amount of $Na_2S_2O_3$ used for the test samples, and the blanks (5 ml HCl and 10 ml water) were recorded and compared with data in a chart of "Somogyi Dextrose Thiosulfate Equivalents".

The formula used for calculating the total sugar was:

$$\% \text{ total sugar} = \frac{\text{Value from chart} \times 100}{\text{Dilution factor} \times 1000}$$

C. pH Determination

Fruits were blended for 1 minute in a Waring Blender. Blended juice was then strained through a double layer of cheesecloth to remove skins and seeds.

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*Solution A: (1) 12 gm Rochelle salt + 20 gm Na_2CO_3 + 25 gm $NaHCO_3$
(2) 6.5 gm $CuSO_4$ $5H_2O$
(3) 10 gm KI + 0.80 gm KIO_3 + 18 gm $K_2C_2O_4H_2O$

The pH was determined with a model 210 Fisher Accumet pH meter. Readings were made on 10 ml samples of juice to which 90 ml of distilled water had been added. Three measurements were taken from each sample.

D. <u>Malic and Citric Acid Determination</u>

Frozen samples were thawed in a manner similar to that used in total sugar determination, but the clear juice of the thawed fruits was used for the paper chromatographic determination of organic acids immediately after thawing without any blending. Slow defrosting resulted in clearer juice, and in this manner filtering was avoided.

Aliquots of clear juice, 10 c.c., from about a 100 gm sample was pipetted into a 25 x 100 test tube and evaporated to dryness over a water bath at about 160^{o} F. A period of about three hours was needed to complete the evaporation.

Residues were taken from the bottoms of the test tubes with a small quantity of 10% isopropyl alcohol and the solutions were made up to 10 c.c. with more alcohol. Samples were stored in this form in vials in a refrigerator until they were needed.

Aliquots of 0.004 c.c. were spotted on 17 inch strips of No. 1 Whatman chromatographic paper, forming as small a spot as possible, about 2 1/2 inches from one end of the paper. The strips were then left to dry.

After air drying the strips ascending chromatography was carried out with butanol : acetic acid : water 12:3:5 (BAW). Development time was approximately 20 hours at 25^{o} C. In order to visualize the spots of malate and citrate, 20 mg of bromophenol blue and 5 mg sodium formate were added to the developing solvent.

The acids appeared as yellow spots against a blue background, and all produced well defined spots with no streaking.

Bromophenol blue had an R_f value high enough to show both malic and citric acids.

In order to establish the identity of the spots of malic acid and citric acid on the paper chromatographic strips, solutions of these two pure acids were prepared and used for a test simultaneously with tomato juice. The strips prepared with tomato juice also showed a few more smaller spots but they were well separated from each other. The identical distances of the pure malic acid and citric acid spots in comparison with the two major spots observed on the strip of tomato juice indicated which of the spots were caused by the malic acid and citric acid content of the tomato sap. The remaining smaller spots on the tomato sap strips were not identified. Later in the work these smaller spots showed a great variation not only in the size of the spots and in their existance but by also being completely missing in the juice of some varieties of tomatoes.

The determination of the spots was carried out with a Model 542 Recording Electrophoresis Densitometer. A 445 m.u. filter was installed and the response knob was set for #8.

To check the validity of densicord readings for comparative evaluation of paper chromatographic malic acid and citric acid spots tests were made of known concentrations. The comparisons were not intended to be a quantitative determination of the two acids, but the values had to be valid for comparative work and to give a clear interpretation about the trend of changes among the different tomato varieties and/or among the different stages of fruit development.

A number of solutions of both malic acid and citric acid were prepared in 4,000 to 100,000 ppm. concentration. Concentrations in a lower than 4,000 ppm. concentration did not give an easily detectable spot on the strip. Triplicate samples of each concentration were used for spotting. All of the strips were put through the Recording Electrophoresis Densitometer. Means of the readings calculated are given in Table 1.

Table 1. Densicord readings of malic acid solutions at 8 different concentrations

Concentration in ppm.	Sample			Average Densicord Reading
	1	2	3	
4,000	21	18	15	18
7,000	25	29	27	27
10,000	35	30	39	35
15,000	42	45	41	43
20,000	61	52	56	56
25,000	62	59	62	61
50,000	72	76	70	73
100,000	96	92	95	94

The table lends itself to the following interpretations:

1. There is a definite relationship among the ppm. concentration of the solutions and the values of the densicord readings.

2. There is a difference between the values of the malic acid and citric acid densicord readings (using a 445 m.u. filter and #8 response) due to the differences in characteristics of the two acids.

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3. The densicord readings increase at a faster rate in the lower ppm. concentration range and this range of sensitivity covers the densicord readings found on the strips which were prepared with tomato sap and presented in tables 7, 8, 12 and 13.

4. The densicord readings do not indicate a quantitative determination of the two main acids but give useful values for the interpretation of the relationship between the two acids and for the trend of relative changes among the different concentrations.

Table 2. Densicord readings of citric acid solutions at 8 different concentrations

Concentration in ppm.	Sample 1	Sample 2	Sample 3	Average Densicord Reading
4,000	16	15	17	16
7,000	27	32	37	32
10,000	35	42	40	39
15,000	51	61	55	56
20,000	67	69	68	68
25,000	67	78	92	79
50,000	110	106	99	105
100,000	109	126	139	125

IV. THE INTERRELATION OF MALIC AND CITRIC ACID, pH AND TOTAL SUGAR TO EARLINESS IN THE FRUITS OF *Lycopersicon esculentum* L.

PART ONE

Detailed Investigations of the Fruits of Three Varieties of Tomato

A. Materials and Methods

Red Bobs, Earlicrop and Starfire varieties were grown for this trial and they were harvested in seven stages of fruit development. The three varieties were grown as part of an advanced varietal trial. The field plan was a randomized block with 4 replications. There were 17 plants per plot.

In a preliminary test to determine the variation in pH in different parts of the tomato fruit, fruits of a limited number of samples were partitioned into locular and pericarp components and analyzed. Equal and opposite sectors of fruits within the samples were partitioned and compared with adjacent sectors blended and analyzed as a whole-fruit sample, to serve as a control. The amount of locular tissue was expressed as a percentage of the fruit weight.

In order to determine the relationship and similarity between fresh and frozen samples of the tomato fruit, another preliminary test was conducted. Fruits of each of the three varieties were tested for pH and total sugar content at seven stages of fruit development, using both fresh and frozen samples. A t-test was applied for analysis of data.

On the basis of results obtained in the two preliminary tests and due to the convenience and great uniformity of treatment, only frozen samples of the three varieties of *Lycopersicon esculentum* L. were analyzed at seven stages of fruit development for pH, total sugar, malic and citric acid.

Correlation coefficients (r) were calculated between the pH and total sugar for each variety and for the average of the three varieties.

Changes in pH and total sugar were studied and correlated to the number of days from transplanting to the first harvest for the varieties tested.

The malic and citric acid were determined in order to ascertain at what stage of development the organic acids appear and disappear in the growing fruit.

B. Results

1. Variation in pH in Different Parts of the Tomato Fruit

The pH of locular tissue was lower than the pH of whole fruit, and the pH of the pericarp tissue was higher than that of the whole fruit or locular tissue (Table 3).

Table 3. Fruit weight, percentage of locular tissue and pH of whole fruit, loculus and pericarp of three varieties of *Lycopersicon esculentum* L. harvested at stage E.

Variety	Fruit wt. in ozs.	% locular tissue	pH		
			whole fruit	locular tissue	pericarp tissue
Red Bobs	3.2	34	4.30	4.24	4.33
Earlicrop	3.6	32	4.30	4.28	4.31
Starfire	4.7	28	4.40	4.37	4.41

Fruits of Red Bobs were slightly smaller and fruits of Starfire were larger than the fruits of Earlicrop. There was also a noticeable difference in the percentage of locular tissue calculated from fruit weight.

The main purpose of the analysis regarding the variation in pH in different parts of the tomato fruit was to establish any relationship in the pH of the various portions of the fruit.

The existance of such a relationship provides us with the opportunity to apply a quick pH testing method in tomato breeding programs. The measurement of locular pH could be carried out in the field with a portable pH meter equipped with a combination electrode and used in conjunction with other selection criteria of the breeding program. Laboratory testing of samples could be limited to plants selected for other desirable characteristics, thus reducing the number of laboratory determinations. Further testing for pH by the more precise, conventional method should be employed in advanced generations.

2. pH of Fresh and Frozen Samples

In the case of the Earlicrop variety the pH was significantly lower at the 1% level, for the fresh samples than for the frozen samples. There were no significant differences between fresh and frozen samples for the other two varieties (Table 4).

3. Total Sugar Content of Fresh and Frozen Samples

The total sugar content of the Red Bobs fruits was significantly lower than for either Earlicrop or Starfire (Table 5). The mean total sugar content of the fresh samples of the three varieties was also significantly lower than in the frozen samples. The means for the fresh samples of Earlicrop and Starfire were also lower than the mean for the frozen samples, although the differences were not statistically significant.

Table 4. pH of fresh and frozen fruits of three varieties of *Lycopersicon esculentum* L. at seven stages of fruit development

Stage of fruit development	pH							
	Red Bobs		Earlicrop		Starfire		Mean	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
A	4.65	4.60	4.67	4.90	4.80	4.80	4.70	4.77
B	4.70	4.50	4.20	4.75	4.91	4.75	4.60	4.67
C	4.35	4.40	4.00	4.70	4.90	4.70	4.42	4.60
D	4.30	4.30	3.80	4.45	4.60	4.65	4.23	4.47
E	4.36	4.30	4.08	4.30	4.40	4.40	4.28	4.33
F	4.37	4.35	4.13	4.40	4.30	4.35	4.27	4.37
G	4.44	4.40	4.24	4.40	4.40	4.40	4.36	4.40
Mean	4.45	4.41	4.16**	4.56	4.62	4.58	4.41	4.51

**Significantly lower (P = 0.01) than frozen fruit.

Table 5. Percent total sugars in fresh and frozen fruits of three varieties of *Lycopersicon esculentum* L. at seven stages of fruit development

Stage of fruit development	Percent total sugar							
	Red Bobs		Earlicrop		Starfire		Mean	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
A	3.70	4.80	3.00	5.20	3.80	4.40	3.50	4.80
B	3.80	5.10	3.50	5.30	3.80	4.50	3.70	4.97
C	4.00	5.40	4.00	5.60	4.00	4.70	4.00	5.23
D	5.00	5.30	6.00	5.40	5.00	6.40	5.23	5.70
F	4.50	5.00	6.50	5.00	6.20	5.50	5.73	5.17
G	4.00	4.90	5.00	4.80	6.00	5.50	5.00	5.07
Mean	4.21**	5.16	4.71	5.30	4.76	5.16	4.56**	5.20

**Significantly lower (P = 0.01) than frozen fruit.

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One possible reason for the higher values from the frozen samples could be the destructive effect of quick freezing on the cell walls. A higher amount of sugar may have been liberated from the cell structure after thawing.

The changes in values among stages appear to indicate a similar trend both for the fresh and frozen samples, therefore it was concluded that frozen samples can be used with satisfactory accuracy for comparative purposes among different varieties and/or among the different stages of fruit development for the determination of pH and total sugar.

4. <u>The Changes in pH and Total Sugar</u>

r value showed a significant negative correlation between pH and total sugar for Red Bobs, Earlicrop and for the total mean of the three varieties. In the Starfire variety the negative correlation between pH and total sugar was highly significant (Table 6).

Table 6. The pH and total sugar of fruit of three varieties of *Lycopersicon esculentum* L. at seven stages of fruit development

Stage of fruit development	Red Bobs		Earlicrop		Starfire	
	pH	Sugar	pH	Sugar	pH	Sugar
A	4.60	4.8	4.90	5.2	4.80	4.4
B	4.50	5.1	4.75	5.3	4.75	4.5
C	4.40	5.4	4.70	5.6	4.70	4.7
D	4.30	5.6	4.45	5.8	4.65	5.1
E	4.30	5.3	4.30	5.4	4.40	6.4
F	4.35	5.0	4.40	5.0	4.35	5.5
G	4.40	4.9	4.40	4.8	4.40	5.5
Mean	4.41	5.16	4.56	5.30	4.58	5.16
r	-0.725*		-0.709*		-0.875*	
r for total mean			-0.493*			

*Correlation is significant at 5% level.

**Correlation is significant at 1% level.

The Red Bobs showed a continuous increase in total sugar and decrease in pH until the green ripening stage (D) of the fruit, followed by a slow decrease of total sugar and an increase in pH. The pattern for the total sugar of Earlicrop was very similar to Red Bobs, but the pH showed a sharper decrease until the early colored ripening stage (E) and a slow increase in later stages. The Starfire showed a peak of total sugar in early colored ripening, Stage E and lowest pH in the stage of late colored ripening (F).

The correlation between the pH and total sugar, and the relationship between the pH value and total sugar content of these three varieties and their earliness is discussed in detail under Part One C.

5. <u>The Changes in Malic Acid and Citric Acid Densicord Units</u>

The two major chromatograph spots tentatively identified as malate and citrate varied considerably in concentration, from 0 to 27 densicord units.

The total of malic acid and citric acid was at the highest level in Stage C and D of fruit development (Tables 7 and 8).

The F value for differences in the malic acid content among the three varieties was not significant (Table 7).

The F value for differences in the malic acid content among the seven stages of fruit development was highly significant (Table 7).

The F value for differences in the citric acid content among the three varieties was significant (Table 8).

The F value for differences in the citric acid content among the seven stages of fruit development was highly significant (Table 8).

Table 7. The malic acid content of three varieties of <u>Lycopersicon esculentum</u> L. at seven stages of fruit development

Stages of fruit development	Densicord Reading			
	Red Bobs	Earlicrop	Starfire	Means
A	13	18	18	16.33abcde
B	19	24	19	20.67ab
C	22	26	18	22.00a
D	26	15	15	18.67abc
E	13	12	12	12.33cde
F	11	0	2	4.33f
G	0	0	0	0.00g
Means	14.86	13.57	12.00	
	a	a	a	

Analysis of variance table

Source	d.f.	M.S.	F
Varieties	2	14.33	0.921
Stages	6	212.98	13.692**
Error	12	15.56	

** Significant at 1% level.

Numbers which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test.

Table 8. The citric acid content of three varieties of Lycopersicon esculentum L. at seven stages of fruit development

Stages of fruit development	Densicord Reading			
	Red Bobs	Earlicrop	Starfire	Means
A	7	10	10	9.00f
B	9	16	17	14.00e
C	13	27	20	20.00abcde
D	16	27	21	21.33abc
E	21	24	22	22.33a
F	18	23	25	22.00ab
G	17	21	24	20.67abcd
Means	14.43	21.14	19.86	
	b	a	ab	

Analysis of variance table

Source	d.f.	M.S.	F
Varieties	2	44.33	3.99*
Stages	6	76.21	6.85**
Error	12	11.11	

* Significant at 5% level.

**Significant at 1% level.

Numbers which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's new multiple range test.

The acid relationship of each of the three varieties was projected on individual graphs as presented in Figs. 1, 2 and 3 (pg. 38, 39 and 40). The changes in malic acid, citric acid and the total of the two acids showed entirely different patterns for the three varieties. The interpretation of these changes is presented under discussion.

C. Discussion and Conclusion

In determining the pH and total sugar of three varieties of *Lycopersicon esculentum* L. at seven stages of fruit development:

1. The author presumed that a negative correlation would exist between the acidity and total sugars of the tomato fruit, i.e., an increase in the total sugar coupled with a decrease in acidity until late colored ripening (F) of the tomato fruit. Chemical analysis showed a somewhat different relationship (Table 6).

Both the sugar and acidity of the Red Bobs variety reached their highest level at Stage D and they both showed a slight but continuous decrease in the later stages.

The pH of Earlicrop was lowest at Stage E and the sugar highest at Stage D.

The Starfire fruit had the lowest pH at Stage F and the highest sugar content at Stage E.

2. Another reason for testing the pH and total sugar level was to determine the possibility of correlation between pH and/or total sugar content of tomato varieties with earliness.

Red Bobs was the earliest of the three varieties, requiring only 65 days to the first harvest. As indicated in Table 4 its mean pH was the lowest among the three varieties, and the lowest pH reading

occurred at Stage D, one stage sooner than for Earlicrop and two stages sooner than for Starfire. This early low in pH value might be considered an indication of early ripening especially in combination with early high in total sugar content at the same stage (D) of development. Mean total sugar of Red Bobs was lower than for Earlicrop and equal to Starfire. The highest sugar content of Red Bobs was lower than in either Earlicrop or Starfire. The significance of such a relationship was not determined statistically at this stage of the investigations.

Starfire was the latest of the three varieties, requiring 87 days to the first harvest. Compared to the earlier Red Bobs, the lowest pH for Starfire occurred at a later stage (F). The highest sugar level for Starfire was also recorded at a later stage (E) than for Red Bobs.

Earlicrop with a requirement of 73 days to first harvest showed a medium mean pH value compared with the other two varieties. The lowest level of pH appeared at an earlier stage (E) than in Starfire (F), but at a later stage than in Red Bobs (D). The peak sugar level was slightly higher for Earlicrop than for Red Bobs, and both peaks were at Stage D. Starfire had the highest sugar content of the three, but it occurred one stage later (E).

On the basis of these observations it would appear that a lower pH value in an early stage of fruit development might be an indication of earliness in a variety. An early high in the total sugar content of a variety may also indicate earliness. The combination of the two characteristics, low pH value in an early stage of fruit development and a variety's highest level of total sugar content being reached at an earlier stage of fruit development may increase the probability of an indication of earliness in a tomato variety.

The pH of each of the varieties decreased during early fruit development and increased during the later stages of maturation.

The total sugar increased during fruit development up to the green ripening stage (D) or to the early colored ripening stage (E) and decreased in the later stages of maturation.

Adequate levels of pH (4.5 or lower) are necessary in processed tomato products to prevent germination of spores of thermophilic organisms not killed or otherwise inactivated by the heating process (39, 44). Higher temperatures and longer processing times may be necessary to provide adequate sterilization when the pH values exceed 4.5. Such excessive processing temperature results in loss of quality in color, ascorbic acid content, texture and flavor. Tomato fruits should have an adequate level of pH (4.5 pH or lower) since organic acids, especially malic acid and citric acid, are also important constituents of tomato flavor (44, 45).

Mechanical harvesting of tomatoes should further increase the need for highest levels of acidity. To obtain high yields from a tomato plant in one harvest, many fruits will have to be left on the vine longer than is necessary with hand harvesting. Since acidity decreases during ripening, it is essential that the potential acid content be high even in the later stages of fruit development.

The acidity problem in tomatoes may be best solved by developing varieties with consistently higher levels of acidity.

High acid lines might be distinguished from standard lines at the early colored ripening stage (E) or at any stage of maturity subsequent to early colored ripening.

The selection of tomato seedlings at Stage (E) coincides with the most important stage of development for commercial harvesting also.

If the tomato is harvested by hand in Stage E, it gives a high grade and very good quality for the fresh market, especially for the tube trade, and it gives the best quality fruits for canning also.

If the tomato is harvested by mechanical harvesters, it is necessary to have a high percentage of total yield in Stage E, in order to gain the highest possible marketable percentage. In this case some of the fruits will reach Stage F, which is still good for local fresh market, or for processing. Some of the fruit will be in Stage D, and these fruits can be used either for fresh market or for processing after post-harvest ripening.

pH varies greatly at different stages of fruit development. However, based on these studies of three varieties it is probably most precisely determined at the early colored ripening stage (E). Selection for acidity at this stage should be less subject to error and more efficient than selections at other stages.

The total acid content of tomato juice is due chiefly to malic and citric acids (24).

On the basis of these present investigations the following observations appear worthy of consideration:

There were noticeable differences among the three tomato varieties in the acid contents of the fruits. The malic acid disappeared in the F and G stage of fruit development (Table 7) and its disappearance appeared to be a specific characteristic of the variety. Citric acid was present in all stages of fruit development (Table 8), but there was a difference among varieties in the quantity and the stage at which the highest level was reached. The total of malic and citric acid was at the

maximum in stages C and D of fruit development. Earlier peak in total acidity may be correlated to earliness in a variety.

In interpreting the acid relationship of the three varieties on their individual graphs (Fig. 1, 2, and 3) it should be noted that the malic acid peak in the Red Bobs tomato and the peak of the total of the two acids were in Stage D (Fig. 1). The malic acid then decreased quickly and disappeared in Stage G. However, it was still at an easily recognizable level in Stage F. The citric acid content was highest at Stage E, (Fig. 1), and then showed a slight decrease towards Stage G. Red Bobs was the earliest of the three varieties.

Koch (27) preferred in his selection program those plants which contained more mean malic acid than mean citric acid in a population. Red Bobs was the highest in the mean of malic acid among the three varieties, and contained more mean malic acid than mean citric acid.

In the case of Earlicrop (Fig. 2) the two acids peaked in Stage C as compared to Stage D in Red Bobs and the total was much higher than for the Red Bobs mainly to the higher citric acid content. These two factors alone as indicated on the previous page are generally related to earliness. However the citric acid content remained on a relatively high level up to the dead ripe stage (G) and such a maintenance of citric acid level may be associated with lateness. Thus accounting for the fact that Earlicrop was eight days later than the Red Bobs. The higher level of citric acid, and the poor malic acid - citric acid ratio in the mean of the two acids, especially in the later stages of fruit development may be associated with the relatively later maturity of the Earlicrop variety compared with Red Bobs.

Fig. 1 Relation of Malic and Citric acids in the Red Bobs tomato at seven stages of fruit development.

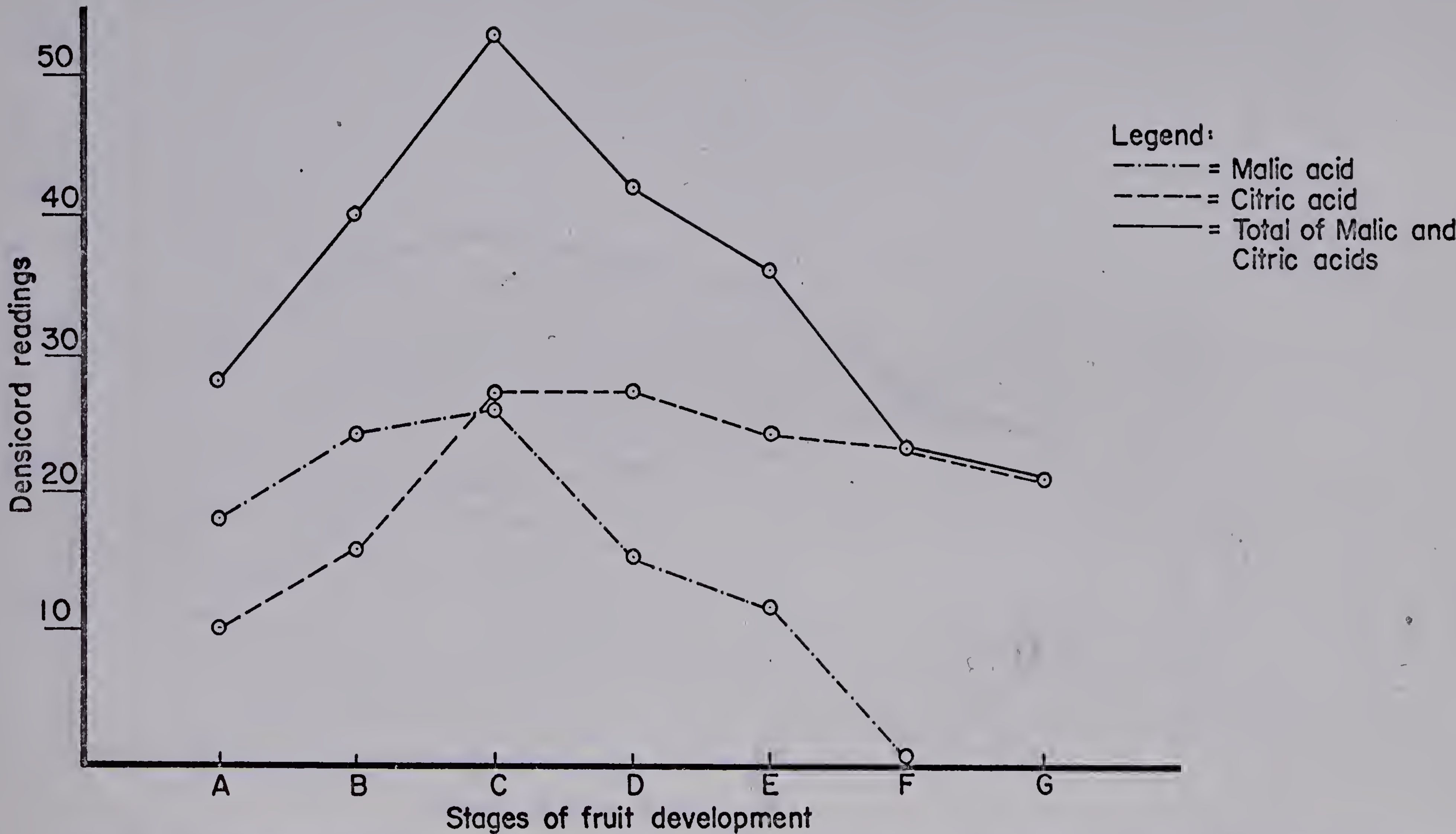

Fig. 2 Relation of Malic and Citric acids in the Earlicrop tomato at seven stages of fruit development.

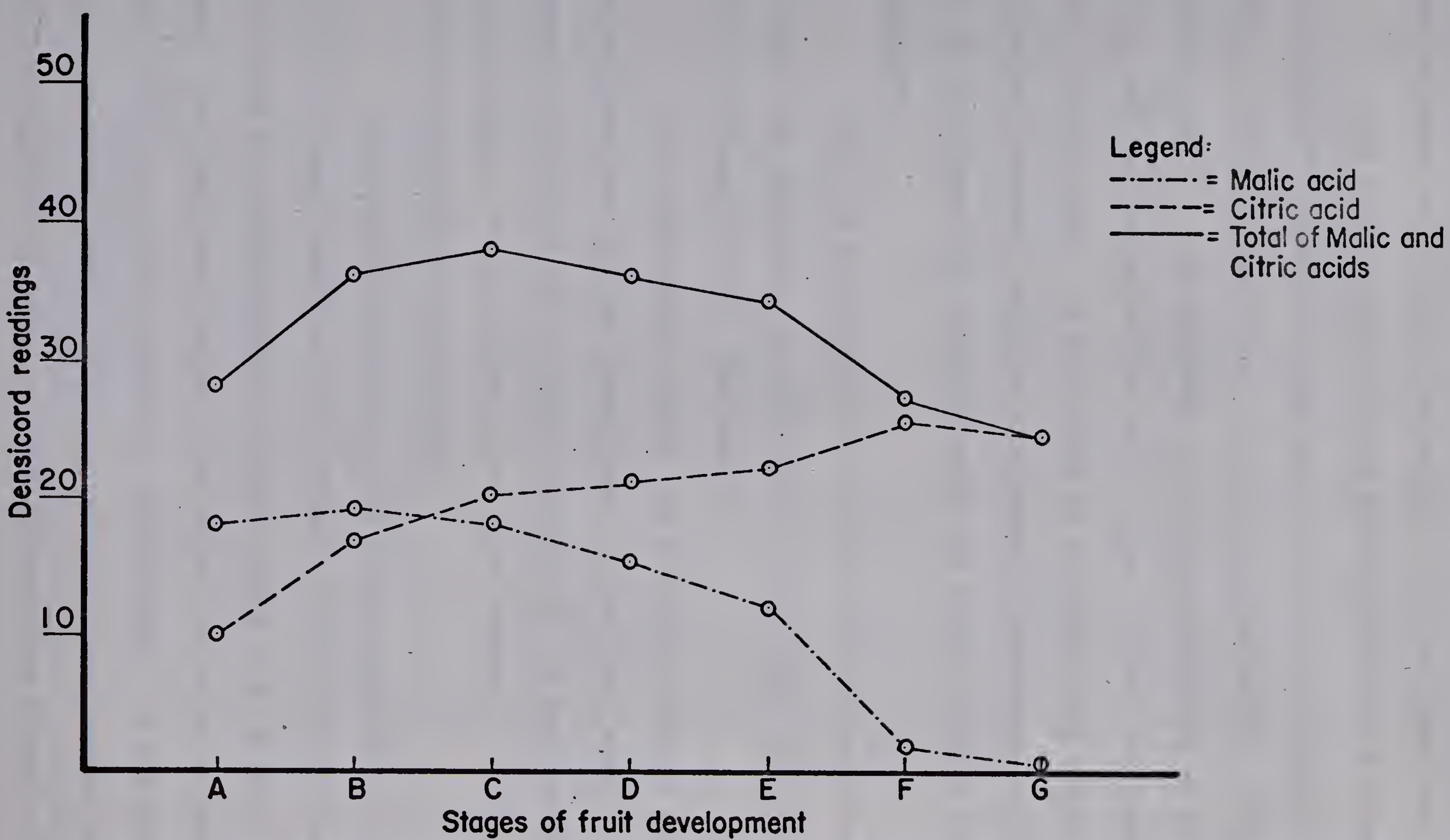

Fig. 3 Relation of Malic and Citric acids in the Starfire tomato at seven stages of fruit development.

The highest malic acid content of Starfire and the mean of malic acid was lower (Fig. 3) than in either Red Bobs or Earlicrop and peaked in Stage B rather than Stage D and Stage C as in the other two varieties.

The total of the two acid in the Starfire was slightly lower than in Red Bobs and much lower than in Earlicrop. Starfire was later than the other two varieties. The outstanding feature of the changes in Starfire was the slow increase in citric acid reading up to Stage F. The citric acid was higher in the later stages of fruit development than in the other two varieties. An acid ratio of low malic acid content and high citric acid content, which was continuously increasing up to Stage F may be associated with Starfire being the latest maturing of the three varieties.

The acid relationship in Starfire variety indicates very strongly the importance of malic acid - citric acid ratio particularly in the later stages of fruit development.

The relationship between the level and ratio of the two acids and earliness was further investigated in Part Two, where a greater number of varieties and a larger variation in the combination of the acid characteristics of the different varieties offered a better opportunity for interpretation and conclusions.

The detailed analysis of the three varieties in seven stages of fruit development indicated that changes in the levels of malic and citric acids were the most meaningful through stages C, D, E, and F. The high peaks, the significant levels of the acids, the relative changes in malic and citric acids appeared in these stages. On the basis of these observations, the number of stages tested for each variety or seedling in the later part of the work was reduced from seven stages to four.

PART TWO

Search for Prospective Parent Material Among Twenty Stocks of *Lycopersicon esculentum* L.

A. Materials and Methods

Varieties and seedlings grown for this project are presented in Table 9. The number of days to first harvest, rating for earliness and growth habit are also listed. The material for this project was harvested in four stages of fruit development. The first, second and seventh stages of fruit development studied in Part One were omitted. It was assumed on the basis of data presented in Part One that the first, second and seventh stages were the least meaningful for checking and interpreting the acid relationship and in the study of the correlation of pH, total sugar, malic and citric acid to earliness.

The early colored ripening stage (E) was assumed to be the single stage of fruit development when the relation of these characteristics would be most meaningful for correlation coefficient calculations. Therefore these calculations were conducted using data from Stage E only. These calculations also included the three varieties previously tested in Part One.

The twenty varieties and seedlings of this trial were grown as part of a single row varietal trial with 17 plants of each. Duncan's New Multiple Range Test was conducted for the grouping of the varieties on the basis of the original triplicate samples of pH, and total sugar.

B. Results

1. The Variations in pH

Samples studied had pH values of 4.07 to 5.50 (Table 10). The

Table 9. Days to first harvest, earliness rating and growth habit of twenty stocks of Lycopersicon esculentum L.

Stock	Days to first Harvest	Rating for Earliness	Growth Habit
B.V. #5	62	very early	determinate
Johnny Jumpup	64	very early	determinate
Rocket	65	very early	determinate
Early North	68	early	determinate
Early Lethbridge	70	early	determinate
Globe Trotter	72	early	determinate
B.V. 2116	72	early	determinate
Tangula	75	midseason	indeterminate
Coldset	75	midseason	determinate
Alpha 5 F_1	76	midseason	determinate
Meteor	77	midseason	determinate
B.V. 2111	78	midseason	determinate
B.V. 2113	78	midseason	determinate
Firesteel	79	midseason	determinate
Early Alberta	80	late	indeterminate
Early Bush Beefsteak	82	late	determinate
Red No. 22	83	late	indeterminate
Earliana	85	late	indeterminate
Rutgers Hybrid	88	very late	indeterminate
Cloche Wonder	91	very late	determinate

Table 10. Days to first harvest and pH in twenty stocks of *Lycopersicon esculentum* L. at four stages of fruit development

Stock	Days to first Harvest	Stage of Development C	D	E	F	Mean
		pH				
Alpha 5 F_1	76	4.60	4.45	4.50	4.52	4.525bc
B.V. 2111	78	4.63	4.62	4.42	4.50	4.543bc
B.V. 2113	78	4.60	4.50	4.40	4.50	4.500bc
B.V. 2116	72	4.80	4.75	4.70	4.75	4.750bc
B.V. #5	62	4.17	4.10	4.07	4.26	4.149c
Cloche Wonder	91	4.50	4.40	4.36	4.90	4.539bc
Coldset	75	4.78	4.81	4.61	4.67	4.717abc
Earliana	85	5.10	4.90	4.70	4.94	4.909abc
Early Alberta	80	4.54	4.52	4.50	4.56	4.429bc
Early Bush Beefsteak	82	5.50	5.40	5.35	5.30	5.375a
Early Lethbridge	70	5.12	5.10	4.80	4.80	4.955abc
Early North	68	5.00	4.92	4.70	4.63	4.813abc
Firesteel	79	4.86	4.80	4.48	4.51	4.663abc
Globe Trotter	72	4.91	4.75	4.37	4.64	4.668abc
Johnny Jumpup	64	4.74	4.70	4.65	4.72	4.703abc
Meteor	77	4.60	4.50	4.40	4.54	4.510bc
Red No. 22	83	4.70	4.62	4.36	4.71	4.597abc
Rocket	65	4.36	4.35	4.41	4.40	4.380c
Rutgers Hybrid	88	4.75	4.40	4.35	4.70	4.550bc
Tangula	75	5.40	4.97	4.97	5.50	5.208ab
Mean		4.781 a	4.678 ab	4.555 ab	4.704 a	

Numbers which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's multiple range test.

Analysis of Variance Table

Source	d.f.	M.S.	F
Blocks	2		
Stages of development (S)	3	0.5270	53.3843**
Error (a)	6	0.0098	
Variety	19	0.9198	91.5969**
Stages x Variety (S x V)	57	0.0419	4.1809**
Error (b)	152	0.0100	

**Significant at 1% level.

differences in pH were highly significant both among stocks and among stages of fruit development. In general, the pH decreased until early colored ripening (Stage E), and then increased in Stage F.

2. The Variations in Total Sugar

Total sugar content of the 20 stocks - tested in four stages of fruit development (Table 11) - varied between 3.00 and 8.20 showing much greater differences between stocks than did pH value. Generally, total sugar reached the highest level in early colored ripening (Stage E), and then decreased with maturity.

The differences in total sugar were highly significant both among stocks and among stages of fruit development.

Table 11. Days to first harvest and percentage total sugar in twenty stocks of <u>Lycopersicon</u> <u>esculentum</u> L. at four stages of fruit development

Stock	Days to first Harvest	Stage of Development				
		C	D	E	F	Mean
		Percent				
Alpha 5 F_1	76	4.00	4.40	6.00	4.00	4.600gh
B.V. 2111	78	4.00	4.17	4.30	4.80	4.317hi
B.V. 2113	78	4.00	4.40	4.60	5.00	4.500h
B.V. 2116	72	4.20	4.57	5.00	4.00	4.442h
B.V. #5	62	3.00	4.20	5.00	4.00	4.050i
Cloche Wonder	91	5.60	6.00	6.50	6.30	6.100b
Coldset	75	4.00	5.40	6.20	6.00	5.399de
Earliana	85	5.60	6.10	6.90	5.60	6.050bc
Early Alberta	80	5.00	5.80	6.00	5.20	5.500de
Early Bush Beefsteak	82	4.20	4.50	5.00	4.30	4.500h
Early Lethbridge	70	4.40	5.20	5.60	6.00	5.300def
Early North	68	4.00	5.00	5.60	5.30	4.975fg
Firesteel	79	5.20	5.90	7.00	6.50	6.149b
Globe Trotter	72	4.40	4.20	4.60	5.40	4.650g
Johnny Jumpup	64	4.00	4.90	5.00	4.70	4.650gh
Meteor	77	4.00	4.20	6.00	4.40	4.650gh
Red No. 22	83	5.20	5.80	6.00	5.60	5.649cd
Rocket	65	4.00	4.20	4.30	4.60	4.275hi
Rutger Hybrid	88	7.00	8.00	8.20	7.80	7.749a
Tangula	75	3.40	5.00	6.20	6.00	5.149ef
Mean		4.460	5.097	5.699	5.275	
		d	bc	a	ab	

Numbers which are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's multiple range test.

Analysis of Variance Table

Source	d.f.	M.S.	Mean
Blocks	2		
Stages of development	3	15.9193	525.6315**
Error (A)	6	0.0302	
Variety	19	9.4891	363.3099**
Stages x Variety (A x B)	57	0.5675	21.7282**
Error (B)	152	0.0261	

**Significant at 1% level.

3. The Variations in Malic Acid

Changes in malic acid levels showed greater variation (Table 12) than pH, sugar content or citric acid level. The samples studied had malic acid densicord readings over the range of 0 to 40 units. The differences in malic acid were apparently significant both among stocks and among stages of fruit development.

Ten stocks reached their highest level of malic acid at Stage C and 7 stocks at Stage D. The mean densicord readings for all the 20 stocks at Stage C and Stage D were 25.40 and 25.30 respectively. The mean malic acid reading decreased sharply in Stage E and in Stage F.

There were five stocks which did not show any malic acid in Stage F. The importance of this fact will be discussed later.

Table 12. Days to first harvest and malic acid densicord readings in twenty stocks of *Lycopersicon esculentum* L. at four stages of fruit development

Stock	Days to first Harvest	Stage of Development				
		C	D	E	F	Mean*
		Densicord Units				
Alpha 5 F_1	76	25	33	15	8	20.25
B.V. 2111	78	23	26	28	11	22.00
B.V. 2113	78	22	30	15	0	16.25
B.V. 2116	72	37	27	19	0	20.75
B.V. #5	62	38	38	17	6	24.75
Cloche Wonder	91	9	10	11	0	7.50
Coldset	75	30	24	12	0	16.50
Earliana	85	15	24	18	7	16.00
Early Alberta	80	26	28	25	17	24.00
Early Bush Beefsteak	82	23	22	14	10	17.25
Early Lethbridge	70	30	40	18	14	25.50
Early North	68	26	20	16	12	18.50
Firesteel	79	16	18	20	9	15.75
Globe Trotter	72	24	33	10	0	16.25
Johnny Jumpup	64	30	16	12	5	15.75
Meteor	77	28	25	23	13	25.25
Red No. 22	83	12	22	21	7	15.50
Rocket	65	26	21	20	16	20.75
Rutgers Hybrid	88	29	24	21	14	22.00
Tangula	75	29	25	18	8	20.20
Mean*		25.40	25.30	17.65	7.85	

*The differences were apparently significant both among stocks and among stages of fruit development.

4. The Variations in Citric Acid

The differences in citric acid were highly significant (Table 13), both among varieties and among stages of fruit development. The variations in citric acid readings were not as great as for the malic acid. Densicord readings for citric acid content varied from 10 to 38.

In general, the peak citric acid content for the stocks in Table 13 was in the green ripening stage (D) and early colored ripening stage (E) with identical means of 23.55 in densicord reading. Means varied from 19.40 to 23.55 compared with the much wider range of 7.85 to 25.40 for malic acid.

Three varieties reached the peak content of citric acid in Stage C: Johnny Jumpup was very early, Coldset early midseason, and Meteor midseason. Meteor reached the peak of 28 in Stage C but the decrease in citric acid content was extremely slow and dropped only to 26 in Stage F. The later maturity of Meteor further enhances the conjecture based on the performance of Earlicrop referred to in Part One.

Four late varieties, Rutgers Hybrid, Early Alberta, Cloche Wonder and Early Bush Beefsteak showed extremely high citric acid content in Stage F. The high citric acid content however was not evident in two other late varieties, Earliana and Red No. 22. Cloche Wonder showed a continuous increase of citric acid level from Stage C to Stage E. Although the malic acid disappeared from Cloche Wonder completely in Stage F, the production of citric acid was so active that the utilization of the citric acid for respiration in the absence of malic acid could not prevent the increment of citric acid in the later stages of fruit development.

Table 13. Days to first harvest and citric acid densicord readings in twenty stocks of <u>Lycopersicon</u> <u>esculentum</u> L. at four stages of fruit development

Stocks	Days to first Harvest	Stage of Development C	D	E	F	Mean*
		Densicord Units				
Alpha 5 F_1	76	22	23	24	22	22.75
B.V. 2111	78	20	26	24	23	23.25
B.V. 2113	78	12	27	19	17	18.75
B.V. 2116	72	19	20	22	21	20.50
B.V. #5	62	23	25	31	24	25.75
Cloche Wonder	91	18	28	24	30	25.00
Coldset	75	27	24	16	15	20.50
Earliana	75	20	22	20	15	19.25
Early Alberta	80	18	25	26	31	25.00
Early Bush Beefsteak	82	26	28	30	29	28.25
Early Lethbridge	70	13	28	25	24	22.50
Early North	68	23	25	29	24	25.25
Firesteel	79	14	18	24	16	18.00
Globe Trotter	72	17	25	24	24	22.50
Johnny Jumpup	64	18	18	15	12	17.67
Meteor	77	28	27	26	26	26.75
Red No. 22	83	11	14	15	14	13.50
Rocket	65	20	21	23	21	21.25
Rutgers Hybrid	88	29	31	34	38	33.00
Tangula	75	10	16	20	18	16.00
Mean*		19.40	23.55	23.55	22.20	

*The differences were apparently significant both among stocks and among stages of fruit development.

Tangula and Johnny Jumpup had a low level of citric acid in all stages of fruit development and both were relatively early.

Although Red No. 22 showed the lowest level of citric acid it was a late variety. Probably the indeterminate growth habit has some tendency to make a variety later compared with similar determinate varieties.

5. The Relationship of Earliness to pH and Total Sugar

Correlation coefficient and regression coefficient calculations were completed between pH and number of days to first harvest and between total sugar, and number of days to first harvest (Table 14).

There was no significant correlation between pH and number of days to first harvest.

The analysis of total sugar and number of days to first harvest gave a signficant positive correlation at the 1% level. The correlation between the total sugar content of the stocks and number of days to first harvest suggests that as a general trend, among the stocks tested a higher number of days to first harvest was required to produce fruits with higher levels of total sugar.

6. The Relationship Between Earliness and Malic and Citric Acid

Correlation coefficient and regression coefficient calculations were completed between malic acid, citric acid, total of malic and citric acid, and number of days to first harvest (Table 15).

The correlations were not significant.

7. The Relative Amounts of Malic and Citric Acids in Four Stages of Fruit Development

The relative amounts of malate and citrate are shown in Figs. 4, 5, 6 and 7.

Table 14. Days to first harvest, pH and total sugar in twenty three stocks of Lycopersicon esculentum L. at stage E of fruit development

Variety	Days to first harvest 1	pH 2	Total Sugar 3
Alpha 5 F_1	76	4.50	6.00
B.V. 2111	78	4.42	4.30
B.V. 2113	78	4.40	4.60
B.V. 2116	72	4.70	5.00
B.V. #5	62	4.07	5.00
Cloche Wonder	91	4.36	6.50
Coldset	75	4.66	6.20
Earliana	85	4.70	6.90
Early Alberta	80	4.50	6.00
Early Bush Beefsteak	82	5.35	5.00
Earlicrop	73	4.30	5.40
Early Lethbridge	70	4.80	5.60
Early North	68	4.70	5.60
Firesteel	79	4.48	7.00
Globe Trotter	72	4.37	4.60
Johnny Jumpup	64	4.65	5.00
Meteor	77	4.40	6.00
Red Bobs	65	4.30	5.30
Red No. 22	83	4.36	6.00
Rocket	65	4.41	4.30
Rutgers Hybrid	88	4.35	8.20
Starfire	87	4.40	6.40
Tangula	75	5.30	6.20
r 1 2		+0.014	
r 1 3			+0.621**
b 1 3			+0.074 percent sugar per days to harvest

**Significant positive correlation at 1% level.

Table 15. Days to first harvest, malic acid, citric acid and total of the two acids in twenty-three stocks of <u>Lycopersicon</u> <u>esculentum</u> L. at Stage E of fruit development

Variety	Days to first Harvest 1	Malic Acid 2	Citric Acid 3	Total of Malic and Citric Acid 4
Alpha 5	76	15	24	39
B.V. 2111	78	28	24	52
B.V. 2113	78	15	19	34
B.V. 2116	72	19	22	41
B.V. No. 5	62	17	31	48
Cloche Wonder	91	11	12	23
Coldset	75	12	16	28
Earliana	85	18	20	38
Early Alberta	80	25	26	51
Early Bush Beefsteak	82	14	20	34
Earlicrop	73	12	24	36
Early Lethbridge	70	18	25	43
Early North	68	16	29	45
Firesteel	79	20	24	44
Globe Trotter	72	10	24	34
Johnny Jumpup	64	12	15	27
Meteor	77	23	26	49
Red Bobs	65	13	23	36
Red No. 22	83	21	15	36
Rocket	65	20	23	43
Starfire	87	12	20	32
Rutgers Hybrid	88	21	34	55
Tangula	75	18	20	38
r 1 2		+0.118		
r 1 3			-0.247	
r 1 4				-0.085

No significant correlation.

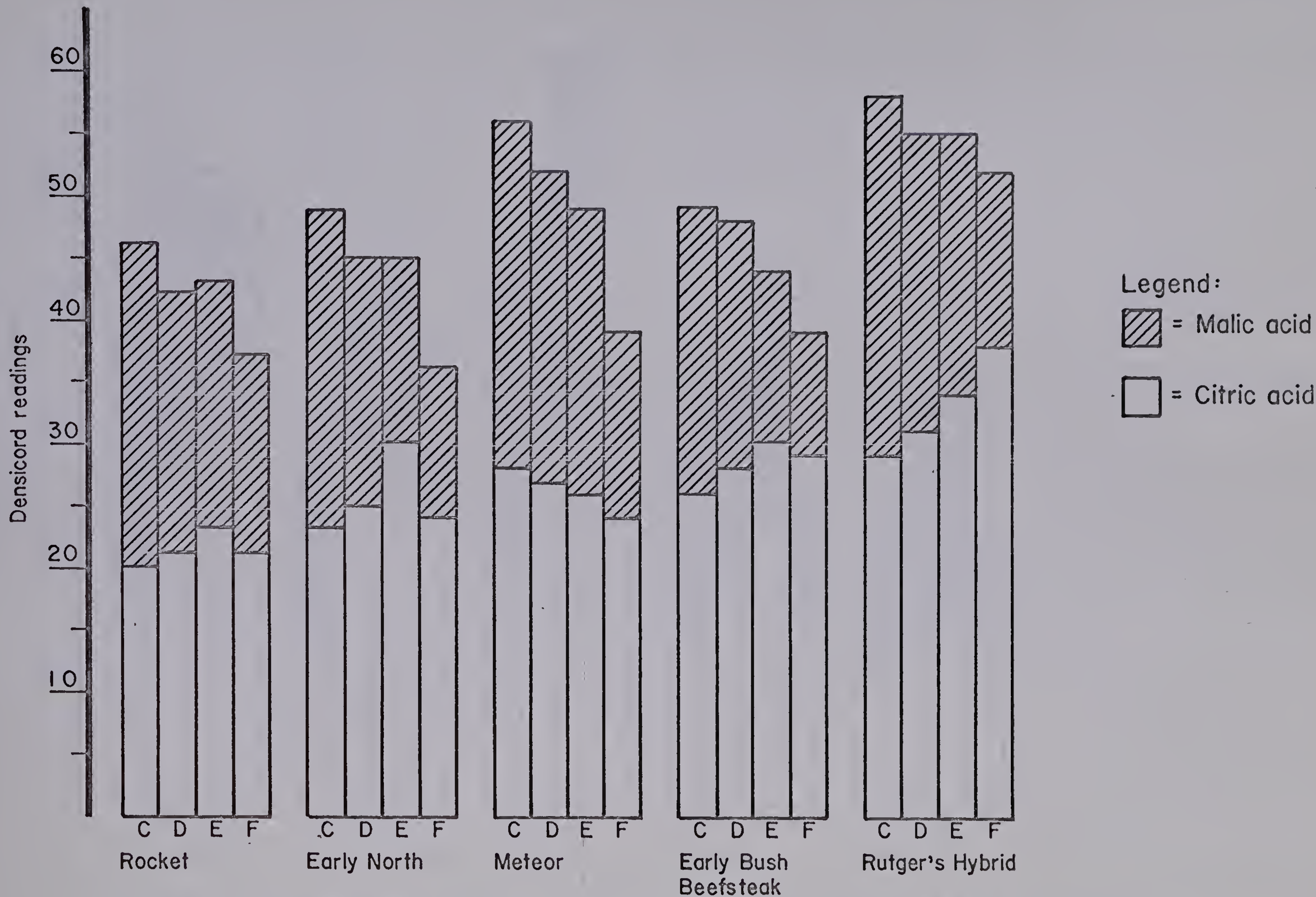

Fig. 4 Relative amounts of Malic and Citric acids in four stages of fruit development.

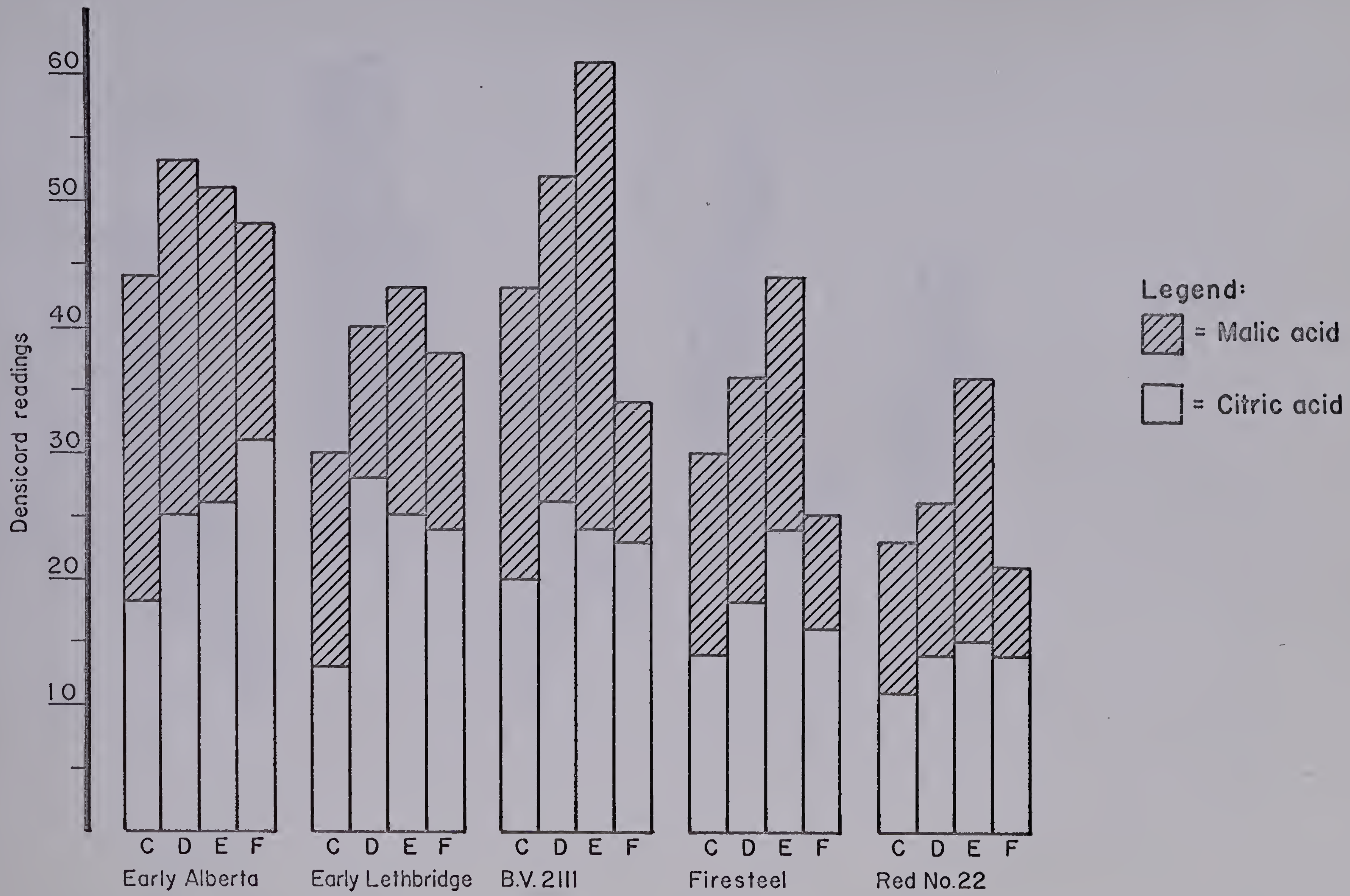

Fig. 5 Relative amounts of Malic and Citric acids in four stages of fruit development.

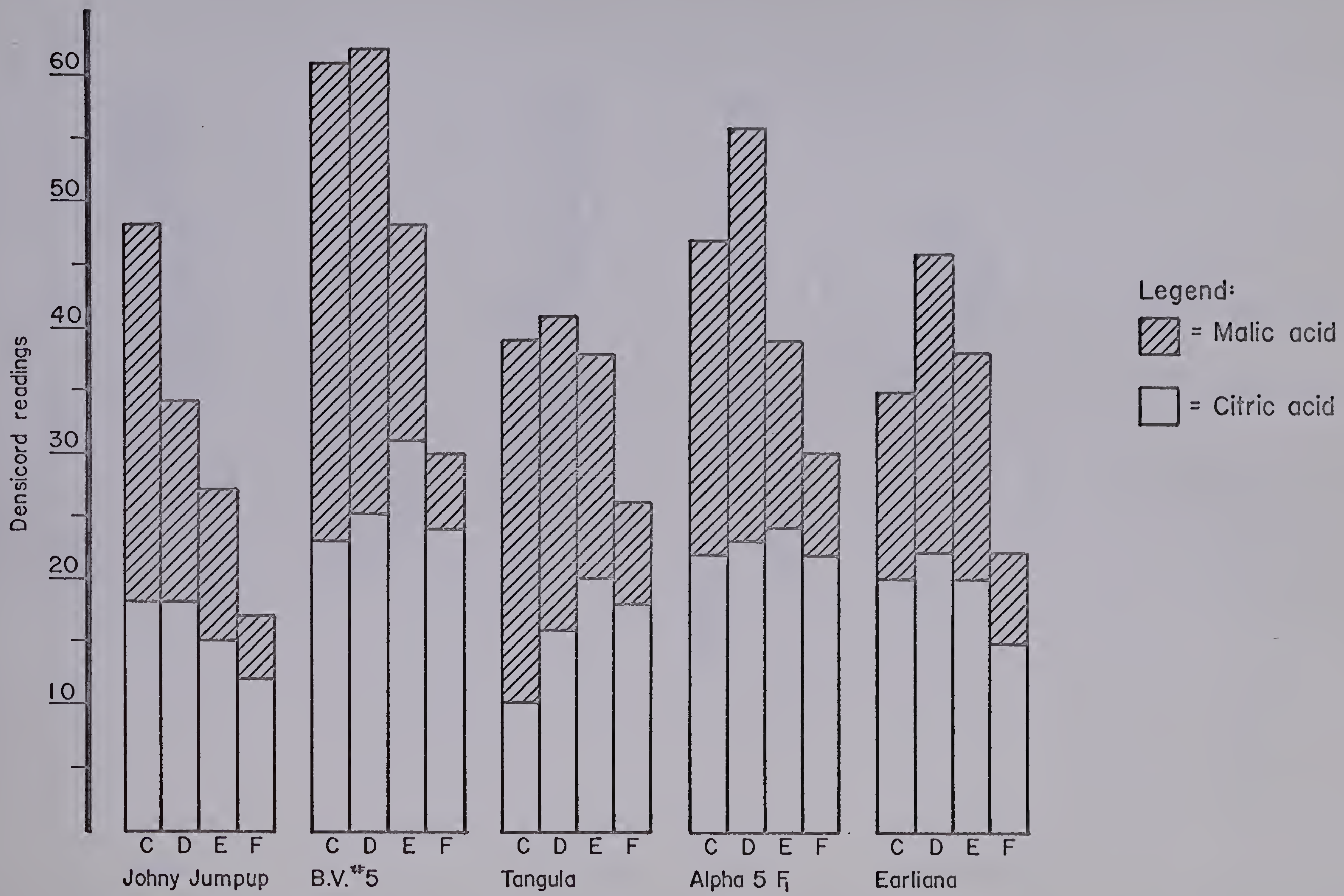

Fig. 6 Relative amounts of Malic and Citric acids in four stages of fruit development.

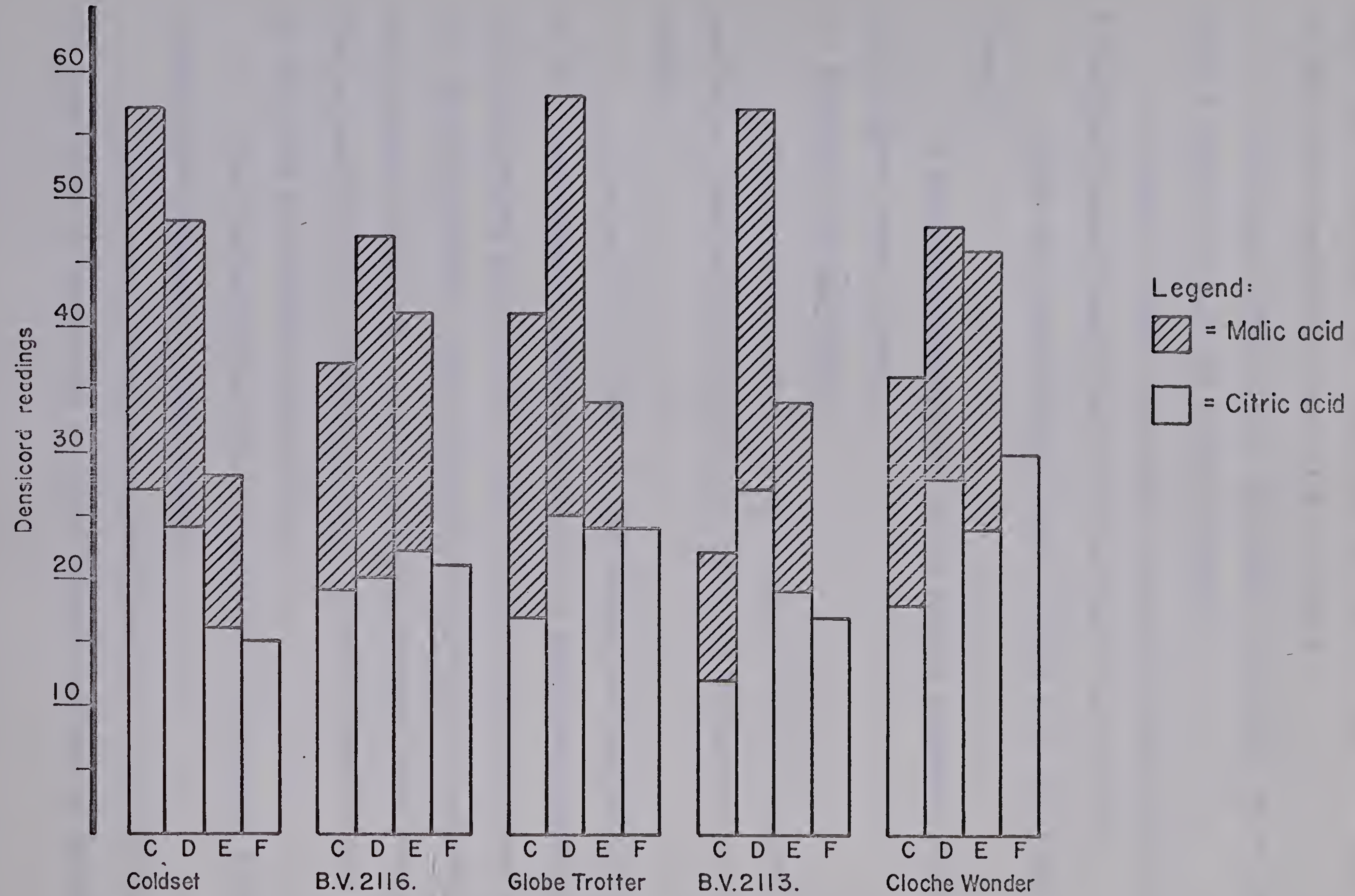

Fig. 7 Relative amounts of Malic and Citric acids in four stages of fruit development.

The five varieties of Figure 4 had both malic and citric acid in all four stages of fruit development and each variety had the highest level of the total of malic and citric acid at Stage C.

In Figure 5 one variety had peaked in the total of two acids at Stage D, and the rest of the stocks in Stage E.

Stocks in Figure 6 had such a low level of malic acid in Stage F that the disappearance of malic acid in Stage G was probable.

There was no malic acid present in Stage F in the fruits of Figure 7.

A detailed interpretation of the results presented in Fig. 4 to 7 is given under discussion.

C. Discussion and Conclusion

The data presented in Table 10 (page 44) did not suggest any particular relationship between the pH value and the earliness of the stocks.

A low pH (4.5 or less) in combination with earliness particularly in the later stages of fruit development is a desirable characteristic from a processing point of view. This valuable combination appeared in B.V. #5 and in Rocket. High acidity in the ripe fruit is extremely important for processing. It cuts down the processing time thus maintaining the quality of the finished product. It usually improves the flavor of the fresh and processed tomato also.

The interpretation of data regarding total sugar in Table 11 suggests some interesting relationships.

Most of the stocks with extremely high total sugar belonged to the late group of tomato such as Cloche Wonder, Earliana and Rutgers

Hybrid, and some of the early stocks showed the lowest level of total sugar, as B.V. #5 and Rocket.

Five stocks peaked in Stage F, and three of them, namely Rocket, Early Lethbridge and Globe Trotter were early varieties.

Globe Trotter, B.V. 2111 and B.V. 2113 are sister selections. The similarity of peak period, level of total sugar and trend of changes for these stocks were marked.

There were a few varieties where earliness was combined with a good level of total sugar - Early North, Early Lethbridge, Tangula and Coldset. This combination could be highly desirable in further breeding programs.

Data presented in Table 12 provide some basis for the following interpretation:

(i) Ten stocks reached their highest level of malic acid in Stage C and 7 of them were early stocks - B.V. 2116, B.V. #5, Early North, Tangula, Coldset, Johnny Jumpup and Rocket. The Early Bush Beefsteak, Meteor and Rutgers Hybird also peaked at Stage C, but Table 11 shows a persistent high level of citric acid for Early Bush Beefsteak and Meteor and the highest level of citric acid for Rutgers Hybrid. The high level of citric acid in these varieties may account for their lateness.

(ii) There were 8 stocks which reached very high densicord readings for malic acid (30, or above) and 5 of the 8 stocks - Globe Trotter, B.V. 2116, B.V. #5, Early Lethbridge and Johnny Jumpup were early; 2 of the 8 stocks - Alpha 5 and Coldset were early midseason and B.V. 2113 was a midseason stock. These observations support Koch's

(27) suggestion regarding the value of high malic acid content of a tomato variety in breeding for earliness.

(iii) There were five stocks which did not show any malic acid in Stage F of fruit development. Three of them - Globe Trotter, B.V. 2113 and B.V. 2116 are descendents of the Cloche Wonder. The fourth, Coldset, is a progeny of Red Bobs which is a sister selection of Globe Trotter. The fifth, Cloche Wonder itself is a late variety under our conditions (high level of citric in the late stages) but it has the characteristic of early disappearance of malic acid associated with earliness. It would appear that this characteristic of early disappearance of malic acid has been transmitted to its progeny.

Changes in malic acid (Table 12) showed greater variation than any of the other characteristics tested. Thus it is suggested that variations in acidity of the tomato may be due chiefly to changes in the malic acid concentration.

It is clear, after detailed study of the existing relationships in Tables 10, 11, 12 and 13, that the correlation between the earliness of different tomato varieties and the biochemical changes of the fruit is a very complex characteristic of the tomato varieties. We believe that the correlation between the earliness of the tomato varieties and the biochemical changes of their fruit is a more complex matter than suggested by Koch (25, 26, 27).

It would appear on the basis of these studies that no single characteristic investigated will by itself serve as an adequate indicator of earliness.

A careful observation of changes and correlations in at least four stages of fruit development is required in predicting earliness. The interaction between malic and citric acids and earliness, and the quantity and ratio of the two acids in certain stages of fruit development may provide a useful relationship.

The five varieties in Fig. 4 had both malic and citric acid in all four stages of fruit development and they all reached the highest total of malic and citric acid in Stage C. Rocket had more malic acid and a little less citric acid than Early North in Stage F. They were both very early varieties: Rocket needed 65 days and Early North 68 days to first harvest. The rest of the varieties in Fig. 4 had higher totals of malic and citric acid and they were all later. One might assume that a high proportion of malic acid in relation to the citric acid is associated with earliness whereas plants with a higher proportion of citric acid may be somewhat later. The citric acid of Meteor peaked in Stage C and it decreased gradually but both the citric acid and the total of the two acids were on a higher level than the previous two varieties. Meteor was a midseason variety, needing 77 days to first harvest. Early Bush Beefsteak contained less malic acid than Meteor but the citric acid peaked in Stage E and it was on a higher level in Stage F than in any of the previous varieties. Bush Beefsteak needed 82 days to first harvest and was rated as a late variety in our classification. The performance of the Early Bush Beefsteak further strengthens the hypothesis that a high level of citric and/or a high proporation of citric acid in relation to malic acid especially in the later stages of fruit development is associated with lateness. Rutgers Hybrid showed the largest total of malic acid and citric

acid in all stages of fruit development. The total of the two acids peaked in Stage C as did all the others in Fig. 4. There was a continuous decrease of malic acid content with a gradual increase of citric acid. The high total acid and high level of citric acid maintained through all stages suggest lateness and the Rutgers Hybrid needed 88 days to first harvest. It was one of the latest stocks tested.

In the Early Alberta variety (Fig. 5) both the malic and the total of malic and citric acid peaked fairly early (Stage D) which would suggest earliness. However, the citric acid increased up to the Stage F. As in Table 8 this increase in citric acid from Stage C to Stage E was associated with lateness. The citric acid level of Early Lethbridge and B.V. 2111 was very similar and the changes paralleled one another in occurrence. The only noticeable difference between the two stocks was the higher level of the total of the two acid in B.V. 2111 till the early colored ripening (E). This factor may contribute to B.V. 2111 being a midseason seedling compared with the early Early Lethbridge.

The pattern of Firesteel was very similar to Red No. 22. A factor which may have influenced the relative lateness of Red No. 22 compared with Firesteel is that Red No. 22 is an indeterminate variety. Observations of other indeterminate varieties showed that they are usually later than determinate varieties with comparable malic acid-citric acid ratios, such as Early Alberta and Tangula.

Varieties in Fig. 6 were grouped on the same graph because of one common characteristic. They all had such a low level of malic acid in Stage F and such a trend in the changes of malic acid content that it made the complete disappearance of malic acid in Stage G probable.

Among these stocks, Johnny Jumpup reached the high peak of malic acid and of the total of the two acids in Stage C. This peak was followed by a continuous decrease to a very low level of the total of the two acids and to the lowest level of citric acid in Stage F of all of the varieties. Johnny Jumpup was one of the very early stocks and needed only 64 days to first harvest.

Earliana reached the peak of malic acid, citric acid and total of the two acids in Stage D. On the basis of densicord readings of acids, trend of changes in acid content, and ratio in the different stages of fruit development, it should be a somewhat earlier variety, but here again an indeterminate variety was tested and compared with determinate varieties.

B.V. #5, Tangula and Alpha 5 are similar in many respects. They each reached the peak of the two acids in Stage D and the peak of citric acid in Stage E, two factors which one might associate with mid-season or late maturity. Tangula and Alpha 5 are midseason varieties with 75 to 76 days required to first harvest. Strictly on the basis of acid relationship, there was no logical explanation for the extreme earliness of B.V. 5 although the high level of malic acid in Stage C and D with a high ratio of malic to citric in these stages as well points toward earliness. One factor should be mentioned. The B.V. #5 is a seedling of Lycopersicon pimpinellifolium L. It might indicate the possibility of an entirely different acid relationship compared with the varieties of Lycopersicon esculentum L.

The stocks of Fig. 7 constituted a separate group with malic acid not present in Stage F as a common characteristic.

B.V. 2116, Globe Trotter and B.V. 2113 are all descendents of Cloche Wonder and the early disappearance of malic acid could have been

inherited from this parent. The increasing citric acid content of Cloche Wonder did not appear in the progenies. One might speculate that the malic and citric acid might be controlled in these crosses by two genes. B.V. 2116 and Globe Trotter were early stocks, both needing 72 days for first harvest. B.V. 2113 was harvested after 78 days. The sharp decrease in citric acid in addition to the disappearance of malic acid of this seedling gave some indication for further potential in earliness. The segregating population of B.V. 2113 should be checked carefully for the appearance of more earliness.

It is suggested that the following factors all play a significant role in relation to earliness of the tomato:

(a) High total sugar content, particularly in the later stages of fruit development is generally combined with lateness. (Starfire in Table 6, Cloche Wonder, Earliana and Rutgers Hybrid in Table 11 and Table 14.)

(b) An early peak of malic acid suggests earliness (B.V. 2116, B.V. #5, Early North, Tangula, Coldset, Johnny Jumpup and Rocket in Table 12).

(c) High densicord reading for malic acid suggests the earliness of a variety. (Red Bobs in Table 7, B.V. 2116, B.V. #5, Early Lethbridge and Johnny Jumpup in Table 12.)

(d) The early disappearance of malic acid (Stage F) is a strong indication of earliness (B.V. 2116, Coldset, Globe Trotter in Table 12.) Selections, where the malic acid would disappear in Stage E would be highly valuable in further breeding programs.

(e) A relatively high content of malic acid to citric acid indicates earliness in a variety. (Red Bobs in Table 7 and 8; Johnny

Jumpup, B.V. #5, Rocket, Early North, B.V. 2116, Early Lethbridge in Table 12 and 13.)

(f) High level of citric acid especially in the later stage of fruit development is a very strong indication for lateness. (Starfire in Fig. 3, Cloche Wonder, Early Alberta, Early Bush Beefsteak and Rutgers Hybrid in Table 13.)

(g) An earlier peak of the total of the two acid and an early peak in citric acid is conducive to earliness. (Johnny Jumpup, Coldset, Meteor in Figs. 4, 6 and 7.)

(h)A later peaking of the total of the two acids usually indicates lateness. (B.V. 2111, Firesteel and Red No. 22 in Fig. 5.)

(i) Late peaking of citric acid and an increase in the level of citric acid is a very strong indication of lateness. (Starfire in Fig. 3, Early Bush Beefsteak, Rutgers Hybrid, Early Alberta, Firesteel, Red No. 22 and Cloche Wonder in Figs. 4, 5, and 7.) The Rocket, Early North, B.V. 2116, B.V. #5, Alpha 5 F_1 and Tangula also showed that characteristic but they were either early or midseason stocks due to influence of the high content of malic acid to citric acid, which indicates earliness of variety.

(j) A continuous decrease in the citric acid reading of a variety during ripening contributes to earliness. (Early Lethbridge, Johnny Jumpup, Coldset and Meteor in Figs. 5, 6 and 7.) The latter two are midseason rather than early varieties probably because of relatively higher levels of citric acid.

PART THREE

The Possible Use of Acid Determination, pH and Total Sugar in the Selection of Tomato Seedlings

A. Materials and Methods

Thirteen seedling selections of B.V. 132-2116-3 were grown to determine within a large number of plants of a promising advanced seedling how suitable acid, pH and total sugar determinations might be in selecting for earliness.

Thirty single plant selections were made from B.V. 2116-3 in the 1965 growing season. The selections were harvested on three dates - August 5, August 15 and August 25. Harvesting was done when three fruits on the plant reached the full ripe stage (F). Selections harvested on August 5 were classified as early (E). Selections harvested on August 15 were classified as midseason (M) and selections harvested on August 25 were classified as late (L).

All the selections were seeded on April 20 in 1966 but the germination and vigor of some of the seedlings was poor, resulting in an insufficient number of some of the selections to be included in 1966 field trial. Seven of the 15 early selections, 4 of the midseason selections, and 2 of the 5 late selections were transplanted to the tomato breeding plot on June 6. Eighty plants of each selection were grown.

The selection of the thirty single plants in 1965 was based entirely on visual evaluation of ripening. Harvesting of the selections in 1966 was done when at least 10 of the 80 plants, all true to type of the seedling, were in the late colored ripening stage (F).

B. Results

1. The Variation in Malic and Citric Acid

Changes in malic acid showed greater variation (Table 16) than the changes in citric acid. The samples studied had a malic acid content of 0 to 30 densicord reading and a citric acid content of 15 to 35 densicord reading. These results were very similar to the observations made in Part Two.

The five selections in Figure 8 contained both malic and citric acid in all four stages of fruit development, and the malic acid level was quite high even in Stage F.

Selections in Figure 9 showed the peak of the two acids in Stage D, but there was a large variation in the peak and quantity of the citric acid and in the relationship and amount of the two acids. In each selection the low level of malic acid in Stage F, and the trend of change in the malic acid content suggested the complete disappearance of malic acid in Stage G.

There was no malic acid present in Stage F in the selections presented in Figure 10.

2. The Variation in pH and Total Sugar

Samples of selections studied had pH values of 4.20 to 5.20 (Table 17). In nine cases the pH decreased until the early colored ripening stage (E), and then showed a slight increase. In 3 cases the lowest level of pH was in green ripening (Stage D), and then increased slowly but continuously. Only one selection(24 M) differed from the general trend of changes of the two previous groups. Selection 24 M had its lowest pH reading in Stage C, and the highest pH in Stage D. Its

Table 16. Malic and citric acid densicord readings at four stages of fruit development in thirteen selections of B.V. 2116-3, and number of days to first harvest

Selection Number	Stage of fruit development								No. of days to first harvest
	C		D		E		F		
	*M	C	M	C	M	C	M	C	
3E	18	27	20	37	22	37	17	28	87
4E	26	19	24	24	13	30	9	32	89
5E	23	29	22	30	13	28	8	26	75
7E	23	17	28	33	22	32	5	29	84
11E	22	35	28	32	18	25	13	23	69
13E	27	22	29	24	26	29	9	26	77
15E	23	20	17	24	5	20	0	16	63
16M	18	24	23	26	10	24	6	22	72
18M	17	20	22	19	6	18	3	15	64
21M	16	15	19	18	10	20	0	18	65
24M	22	16	20	21	12	24	0	18	68
27L	24	20	30	23	16	20	9	15	71
28L	20	21	24	24	21	26	17	24	73

*M = malic acid

C = citric acid

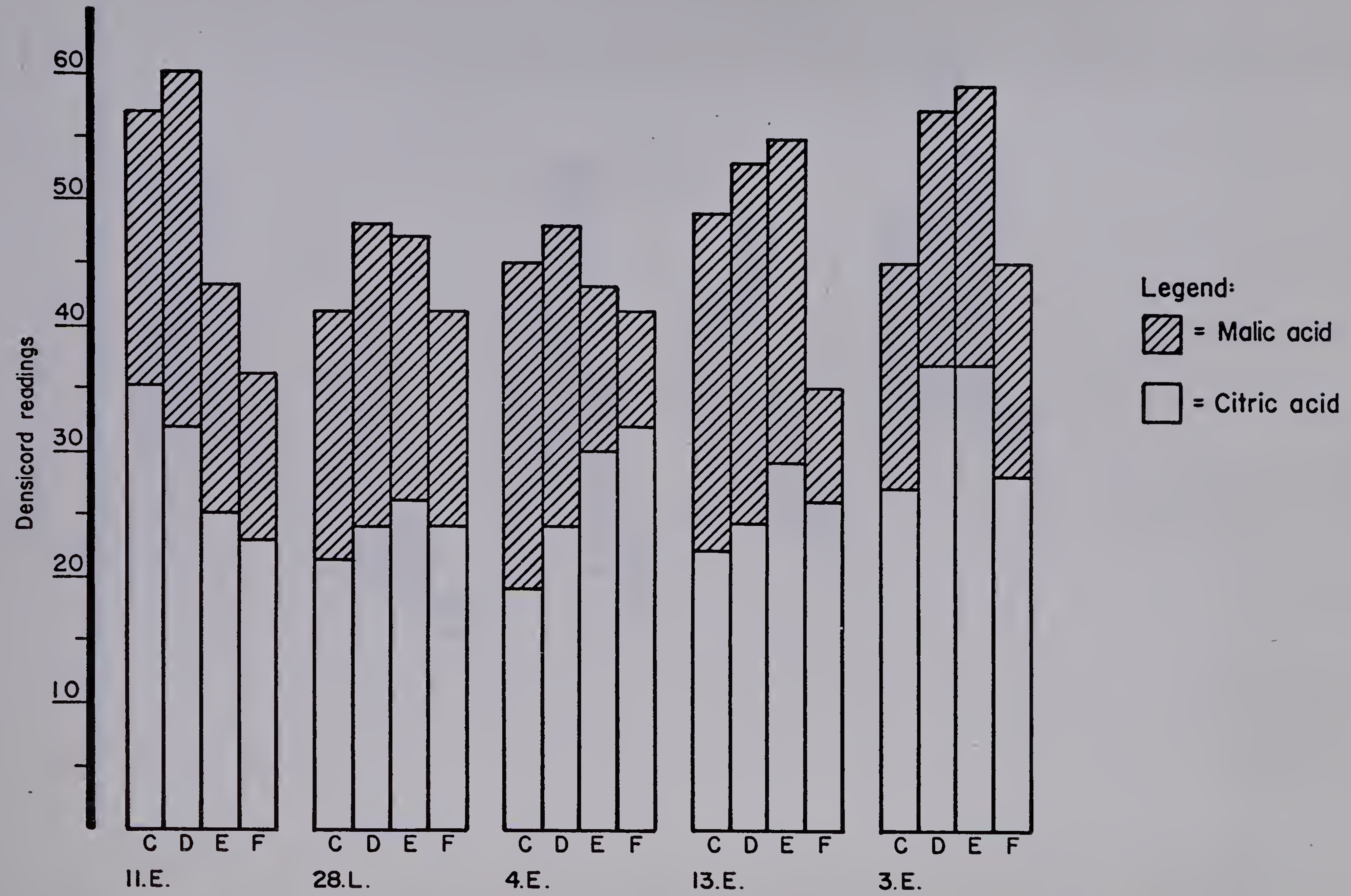

Fig. 8 Relative amounts of Malic and Citric acids in four stages of fruit development.

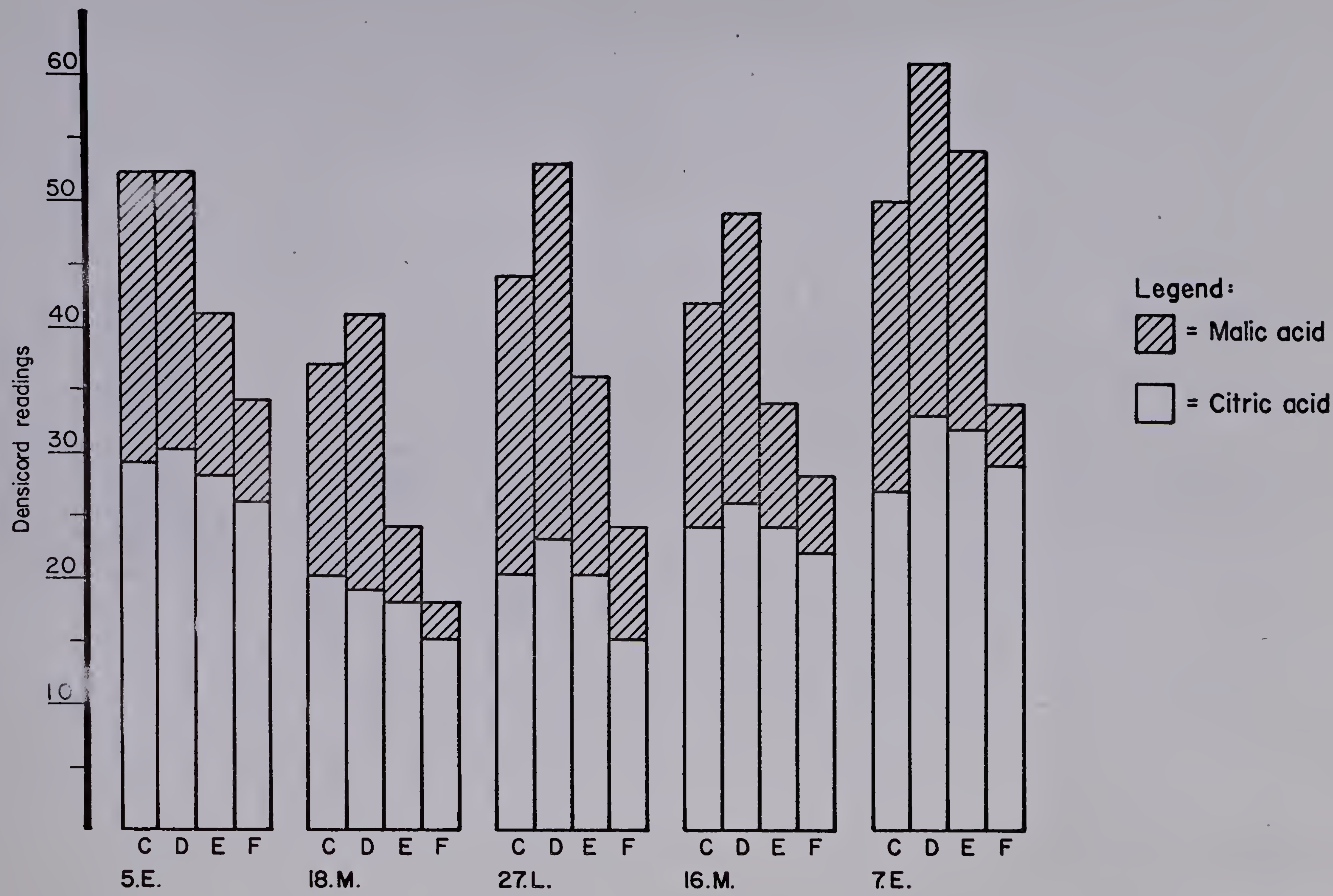

Fig. 9 Relative amounts of Malic and Citric acids in four stages of fruit development.

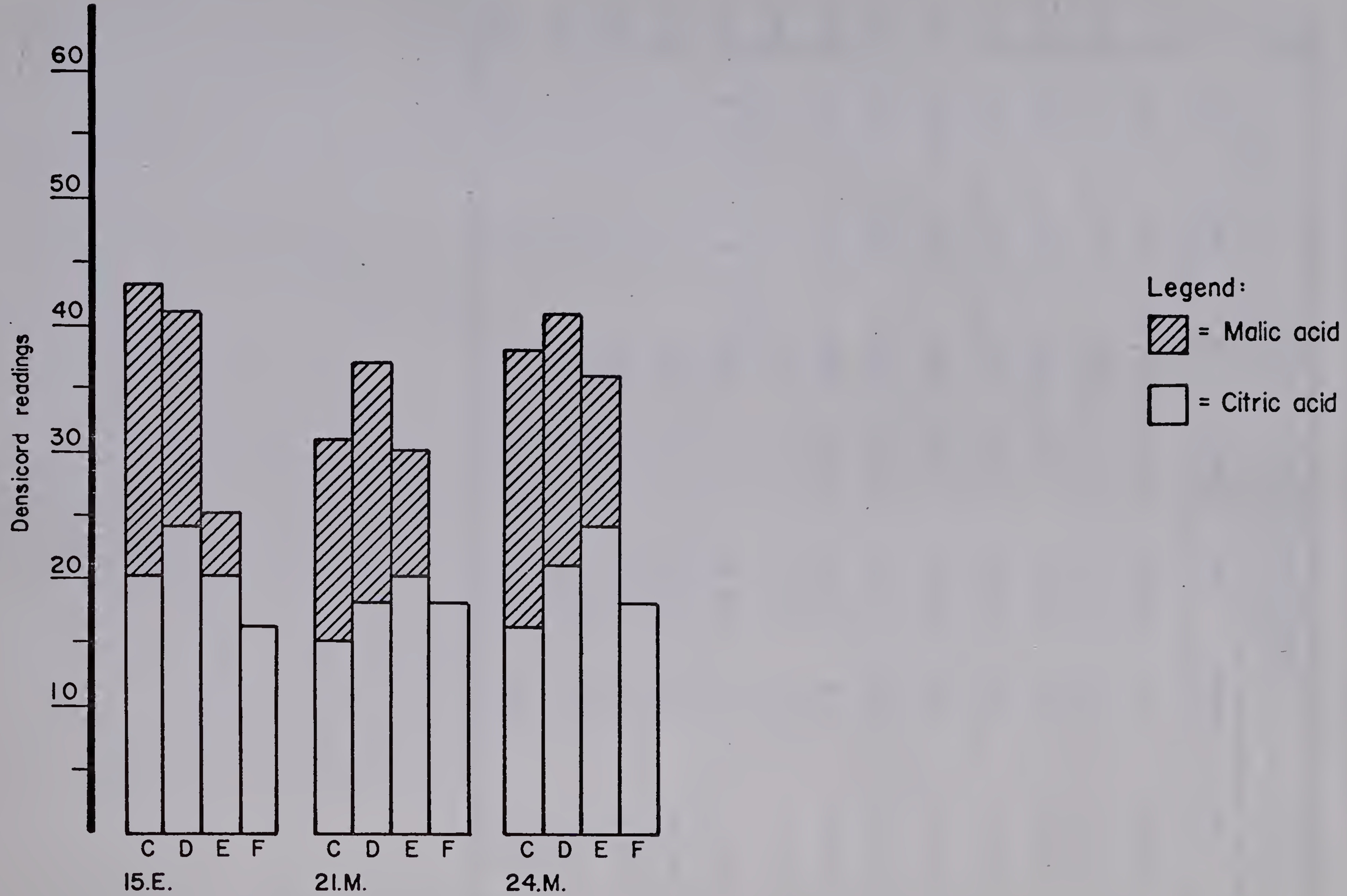

Fig. 10 Relative amounts of Malic and Citric acids in four stages of fruit development.

Table 17. The pH and percentage total sugar at four stages of fruit development in thirteen selections of B.V. 2116-3

2116-3	Stage of fruit development							
	C		D		E		F	
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar
3E	4.70	3.6	4.60	5.1	4.52	5.2	4.76	4.4
4E	4.92	4.0	4.56	5.0	4.58	5.0	4.64	3.8
5E	4.90	4.1	4.85	5.1	4.86	4.9	4.90	4.1
7E	4.90	4.2	4.65	5.5	4.64	4.9	4.70	4.3
11E	4.60	3.8	4.42	4.0	4.54	4.3	4.68	3.9
13E	4.75	3.5	4.55	4.0	4.50	4.4	4.60	3.6
15E	4.95	4.6	4.86	5.4	4.60	5.1	4.80	4.2
16M	5.12	3.8	5.09	4.9	4.89	5.9	4.91	4.9
18M	5.20	3.9	5.00	5.0	4.82	5.6	5.00	4.7
21M	4.90	4.0	4.90	4.2	4.65	4.7	4.70	4.2
24M	4.20	4.5	5.00	5.4	4.75	4.1	4.95	3.6
27L	4.98	3.7	4.74	4.0	4.60	4.5	4.65	4.4
28L	4.90	4.0	4.70	4.8	4.40	4.5	4.50	4.2

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pH value decreased in Stage E and increased again in Stage F. It is possible, that due to the shading effect of some exceptionally heavy foliage, the color development of this sample was actually behind the true stage of ripening, resulting in a high pH value.

The total sugar content of the 14 selections varied between 3.5 and 5.9, showing greater difference than the pH value. The total sugar reached the highest level at Stage D in 6 cases, at Stage E in 7 cases, and decreased with further maturity.

The lowest pH reading and the highest total sugar content occurred in the same stage of fruit development in 8 cases. The highest sugar was shown one stage before the lowest pH four time, and twice the lowest pH was read two stages before the high peak of sugar.

C. Discussion and Conclusion

The first three selections in Figure 8 reached the peak of the total of malic and citric acid in Stage D but the citric acid densicord reading of the same selections showed an entirely different picture.

In 11E the citric acid peaked in Stage C and from there on decreased very rapidly, a characteristic associated with earliness. 11E required 69 days to first harvest. It is a low pH and low sugar selection.

The peak of citric acid for 28L appeared at Stage E. The total of the two acids was still high in Stage F, but nearly half of the total of the two acids was malic acid. On the basis of visual selections in 1965 this selection was judged to be late. In 1966 it was classified as midseason with 73 days needed to first harvest. This seedling must be checked carefully in the following years. It is felt that the actual number of days to first harvest was less than the real potential of the

seedling. The slow increase in the citric acid level up to Stage E would suggest lateness, but the high densicord reading for malic acid indicates earliness. The two situations constitute a rare combination of two characteristics which effect the seasonability of tomato varieties in opposite ways.

In 4E the citric acid increased up to the highest level in Stage F. This selection was the latest of those tested. This characteristic of citric acid relationship is in full agreement with the observations made in Part Two.

The last two selections on Figure 8 peaked in the total of the two acid in Stage E, with quite high totals. 13E was classed as a midseason seedling from an early selection based on 77 days required from transplanting to harvest. It showed a late peak (Stage E) of citric acid, and poor malic acid-citric acid ratio. The citric acid showed a much higher level in 3E and was the second latest selection. Low pH was combined with poor total sugar.

The first selection in Figure 9, 5E, peaked in the total of the two acids at Stage C and at Stage D for citric acid. The citric acid was on a relatively high level, but due perhaps to the gradual decrease of the citric acid, from an early stage of fruit development, 5E performed as a midseason selection requiring 75 days to first harvest. High total sugar in the early stages and high pH in the late stages of development gave a poor combination for quality.

All other selections of Figure 9 showed the peak of the two acids in Stage D but there was a large variation in the peaks and quality of the citric acid and in the relationship of the two acids.

18M was high in malic acid in the early stages of fruit development, but it almost disappeared in Stage F. The citric acid peaked in Stage C and it was low at each stage. The ideal malic-citric relationship made it one of the earliest selections. According to our observations, made in Part Two, the high malic acid in relation to low citric acid readings in the early stages of fruit development, continuous decrease of the citric acid from Stage C, and the quick disappearance of the malic acid all support this earliness. The high total sugar at Stages E and F is an additional favorable factor in considering the value of this selection. The trend of changes for total acid was very similar for 27L and 16M. Although the total of the two acids was a little higher for 27L in the early stages of fruit development, it is felt that on the basis of low citric acid levels in identical stages, and faster decrease of citric acid level this seedling has even more early potential. The low pH - high sugar combination in Stage F was very desirable.

The peak of citric acid for 7E was at Stage D, but it remained on a high level in all stages. The high citric acid, and the high total of the two acids, caused the lateness of this selection. It was good in total sugar.

In the selections of Figure 10 the malic acid disappeared in Stage F. The changes of citric acid, and the different malic-citric relationship can be the reason for the slight differences in the number of days to first harvest, but they all were very early or early selections. The 15E selection was earliest with 63 days to first harvest. The high total sugar content was an additional good characteristic of this selection. Although 21M and 24M exhibited the same characteristics associated

with earliness as did 15E, the mediocre pH and total sugar levels of 21M and the high pH and low sugar of 24M would render them undesirable in a breeding program.

The 1965 selections showed large variation in earliness in the 1966 season.

The visual selection of breeding material for earliness was not a reliable method due to human factors involved in the selection and due to the effect of seasonal climatic conditions, which greatly influenced the development and performance of the same selections during the growing season.

The detailed analysis of the malic and citric acids, was a lengthy procedure and required much careful consideration. However the changes of the two acids and the study of the pattern of the acid relationship appeared to be a useful tool in selecting for earliness.

According to Koch (27) a shorter ripening period of the tomato is dependent upon the presence and quantity of malic acid. He (26) suggests, the number of days required to ripen tomato fruit can be decreased either by increasing the quantity of malic acid present in the fruit, or by decreasing the quantity of citric acid.

The author suggests that in breeding for earliness it may prove useful to select for the following characteristics:

- high level of malic acid
- early disappearance of malic acid
- high proportion of malic acid compared with citric acid
- low level of citric acid in all stages of fruit development
- early peak of the citric acid

- early peak of the total of the two acids
- a rapid decrease of citric acid levels from an earlier stage of fruit development.

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